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EFFECT OF TOPICAL TINOSPORA CORDIFOLIA ON HEALING OF BURN WOUNDS IN WISTAR RATS

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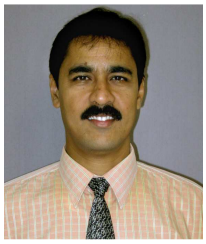
ABSTRACT

Objective: To investigate the effect of tinospora cordifolia (TC) on the healing of burn wounds in wistar rats and compare it with control and silver sulfadiazine treated groups. Materials and Methods: Partial thickness burn wounds were made on three groups of rats (n=6 in each group) at the nape of neck under ether anesthesia. The Burn wounds were treated topically with petroleum base, 1% silver sulfadiazine and 2% TC, respectively once daily till complete healing. The wound contraction rate and epithelialization time were observed. Data was analysed by one way ANOVA and Tukeys multiple post hoc procedures. Results: The mean epithelialization time was significantly ($P<0.0004^*$) decreased in TC treated group compared to control and silver sulfadiazine groups. Percentage of wound contraction was significantly more on 4th ($P<0.0003^*$), 8th ($P<0.0019^*$), 12th ($P<0.0060^*$) and 16th day ($P<0.0016^*$) in TC treated group in comparison to the control. Conclusion: Topical TC showed healing potential on burn wounds in wistar rats.

KEY WORDS: Burn wound, Tinospora Cordifolia, Epithelization, Wound contraction



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INTRODUCTION

'Burn' can be defined as tissue damage caused by a variety of agents such as heat, chemicals, electricity, radiation etc. The most common are burns caused by scalds, fires, flammable liquids etc. Burn injuries to the skin result in loss of its protective function as a barrier to micro-organisms leading to the high risk of infection [1]. Thus, burn patients face high morbidity and mortality because of the large uncovered burn surface getting infected, healing of which takes long periods of dressings, leading to deformities and contractures [2]. Every year, about two million people receive medical treatment for burn injury [1]. The management of the burn wounds still remains a matter of debate and the outcomes of existing methods are far from optimal. Hence, there is need for safer and effective burn wound-healing agents.

In 'ayurveda' (an ancient Indian system of medicine) several indigenous drugs have been described for the management of wounds and burns, but they lack enough scientific data to support the claims made in ancient literature. *Tinospora cordifolia* (TC) [3] is one such agent used in the ayurvedic medicines since time immemorial for the treatment of various conditions viz; jaundice, fever, diabetes, skin diseases, as an antiseptic etc. This has not been subjected for scientific evaluation on burn wound healing. In the present time Tinospora cordifolia has been subjected for numerous chemical, pharmacological, preclinical and clinical investigations and many interesting findings are reported including *antimicrobial* [4], *antioxidant* [5], *antidiabetic* [6] and *immunobiological activities* [7]. The various extracts of TC exhibited antimicrobial activity against various microbes including those causing burn and wound infections like *pseudomonas aeruginosa* and *staphylococcus aureus*[8], [9]. Hence, TC could be beneficial in the management of burn wounds

In view of paucity of information of TC on burn wound healing, the study was undertaken with the aim of investigating the influence of topical application of TC on burn wounds as well as to compare it with control and silver sulfadiazine treated groups in wistar rats.

MATERIALS AND METHODS

The study was conducted after obtaining the approval of the Institutional Animal Ethics Committee.

Animals: Healthy male Wistar rats weighing between 180-200 g were used in the study. They were housed individually and maintained on normal diet and water *ad libitum.*

Study design: The animals were randomly allocated into three groups of six animals each. Group-I control (petroleum base), Group-II standard (1% silver sulfadiazine ointment) and Group-III test (2% tinospora cordifolia cream, courtesy M/s Himalaya pharmaceutical company, Bangalore).

Dosing schedule: Drugs were administered topically, once daily from day 0 to the day of complete healing or the 21st postoperative day, whichever was earlier.

Wound model: Overnight starved animals were inflicted with partial thickness burn wounds under ether anesthesia, by pouring hot molten wax at 80°C into a metal cylinder with 300 mm^2 circular opening, placed on the shaven back of the animal [10].

The parameters were observed during study as follows:

a) *Epithelialization period*: It was monitored by noting the number of days required for the eschar to fall off from the burn wound surface without leaving a raw wound behind.

b) *Wound contraction*: It was noted by following the progressive changes in wound area planimetrically, excluding the day of the wounding. The size of the wounds was traced on a transparent paper every two days, throughout the monitoring period. The tracing were then transferred to 1 mm^2 graph sheet, from which the wound surface area was evaluated. The evaluated surface

area was then employed to calculate the percentage of wound contraction, taking the initial size of the wound, 300 mm^2, as 100%, by using the following equation:

Percentage of wound contraction

$$= \frac{\text{1- Wound area on corresponding day}}{\text{Wound area on day zero}} \times 100$$

STATISTICAL ANALYSIS

All results are expressed as the mean ± SD. One way ANOVA was applied for testing the significant difference between three groups at each time point. Tukeys multiple post hoc procedures was applied to see the significant difference between pairs of two groups.

RESULTS

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

In control animals wound contraction was to the extent of 14%, 27%, 40%, 59% and 78% by day 4, 8, 12, 16 and 18 respectively. These animals took 29 days for complete epithelization. While, topically applied 2% TC shortened the period of epithelization significantly (*P=0.0004) by 8 days (*Table.2& Figure.2*). Besides, it also promoted the wound contraction throughout the study (*Table.1& Figure.1*) as compared to the control and standard groups. There was a statistically significant (*P=0.0028) reduction of scar area in TC group as compared to that of control (*Table.2& Figure.2*). Topical 1% silver sulfadiazine did not affect the healing of excision wounds significantly as compared to control (*Table.2& Figure.2)*. However, there was a significant (*P=0.0493) reduction in the epithelization time to 4 days as compared to control. The percentage of change in the healing of burn wounds from 4^{th} to 18^{th} post wounding days was compared in all the three groups and it was observed significant changes in all the three treated groups.

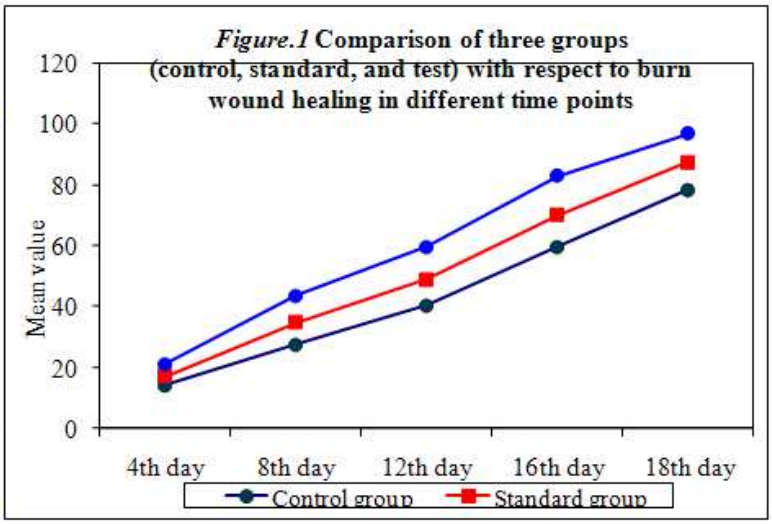
It is evident from these findings that the topical application of TC enhances the healing of burn wounds. However, in the present study the effects of silver sulfadiazine on the wound contraction were not statistically significant.

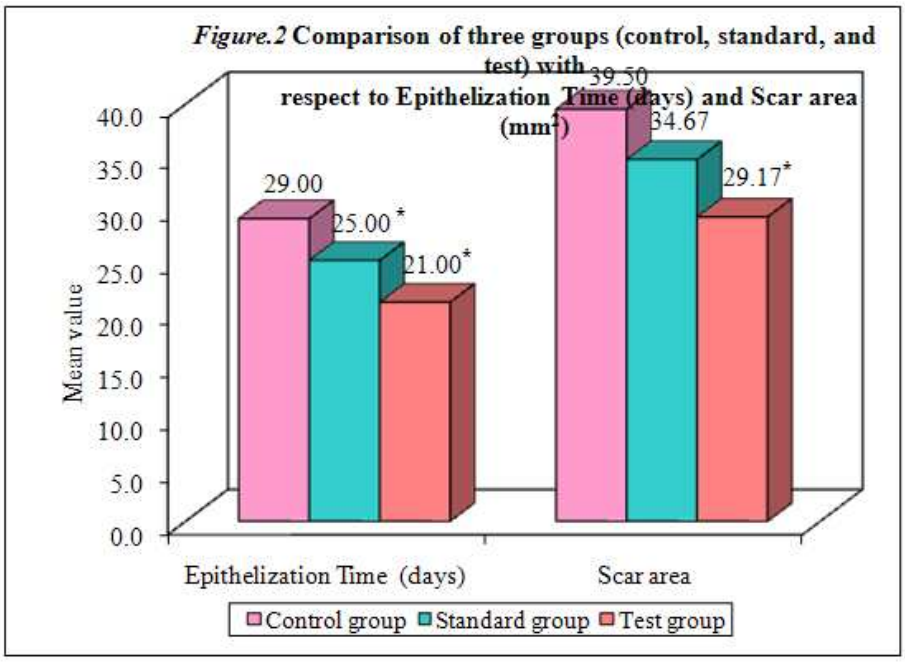
Table: 1

Comparison of three groups (control, standard, and test) with respect to burn wound healing in different time points

Groups (n=6 in each)	Summary	Wound closure rate (%)					% of changes of healing from 4th day			
		4th	8th	12th	16th	18th	4-8	4-12	4-16	4-18
C	Mean	14.09	27.50	40.45	59.43	78.46	13.40	26.36	45.34	64.37
	SD	1.85	4.14	4.46	8.48	8.14	2.49	3.05	6.95	6.68
S	Mean	17.04	34.64	48.87	70.14	87.52	17.60	31.83	53.10	70.48
	SD	2.16	6.12	10.55	10.54	7.26	4.11	8.59	8.59	5.71
T	Mean	21.15	43.53	59.82	83.08	97.05	22.38	38.66	61.93	75.90
	SD	2.46	8.42	10.84	8.91	3.54	6.06	8.48	6.53	1.39
C	-	-	-	-	-	-	95.09*	187.03*	321.7*	456.7*
S	-	-	-	-	-	-	103.3*	186.81*	311.6**	413.6*
T	-	-	-	-	-	-	105.8*	182.79*	292.8	358.8*
F-value	-	16.00	9.252	6.819	9.625	11.84	6.0768	4.4109	7.540	7.5773
P-value	-	0.0002*	0.0024*	0.0078*	0.0020*	0.0008*	0.0117*	0.0311*	0.0054*	0.0053*
Pair wise comparisons of three groups by Tukeys HSD										
C vs S (p-value)	-	0.079	0.169	0.275	0.150	0.076	0.264	0.407	0.1984	0.1317
C vs T (p-value)	-	0.0003*	0.001*	0.006*	0.001*	0.0007*	0.008*	0.024*	0.004*	0.0040*
S vs T (p-value)	-	0.0133*	0.0751	0.1276	0.0727	0.0608	0.1866	0.2580	0.1311	0.1945

(Note: *p<0.05 C- control, S-standard, T-test)





*P<0.05

Table: 2

Comparison of three groups (control, standard, and test) with respect to Epithelization Time (days) and Scar area (mm^2)

Groups	Summary	Epithelization Time (days)	Scar area (mm^2)
Control	Mean	29.00	39.50
	SD	2.00	5.24
Standard	Mean	25.00	34.67
	SD	3.63	4.55
Test	Mean	21.00	29.17
	SD	2.00	3.13
F-value	-	13.5849	8.3055
P-value	-	0.0004*	0.0037*
Pair wise comparisons of three groups by Tukeys HSD			
C vs S (p-value)		0.0493*	0.1716
C vs T (p-value)		0.0004*	0.0028*
S vs T (p-value)		0.0493*	0.1098

*p<0.05

Note:

1. ***One way ANOVA was applied for testing the significant difference between three groups at each time point***
2. ***Tukeys multiple post hoc procedures was applied to see the significant difference between pairs of two groups***

DISCUSSION

The present study was designed to investigate the influence of the tinospora cordifolia (TC) on experimentally induced burn wound in wistar rats. The result demonstrates and confirms the efficacy of TC on burn wound healing and thereby proves the folklore use of the herb for variety of wounds. 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.

The TC was found to be effective at mainly three main phases of wound healing, i.e. Collagenation, wound contraction and epithelization. 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 burn wound model, clearly indicate that TC has promoted the healing of burn 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. Furthermore, TC hastened the period of epithelialization significantly as compared to that of control and standard. It appears that TC has potential 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.

As our (by the same authors) previous studies reported that TC applied locally or administered orally has promoted the breaking strength, wound contraction, period of epithelization and hydroxyproline concentration in different wound models [13], [14]. In the present study, the pro-healing effect of TC in burn wounds corroborates with our previous study report that topical TC would promote contraction and epithelization of excision wounds [13].

The present study was not aimed at exploring the mechanisms for the pro- healing effects of TC. However, the pro-healing effect of TC could be attributed to its known *antimicrobial* [4], *antioxidant* [5], *antiinflammatory* [15 & 16] and *immunobiological* [7] activities. These various activities may play an important role in wound healing.

Infection is one of the most common complications of burns which interfere with healing of wounds [17]. Infection in wound is due to the presence of necrotic tissue and disruption of protective barrier to micro-organisms. 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. [18] TC has an antimicrobial activity against various microbes including those causing burn and wound infections like *pseudomonas aeruginosa* and *staphylococcus aureus* [8], [9]. Consequently, it would be expected to produce pro-healing effect on infective burn wounds. However, in the present study the wounds were appeared clean and healthy; therefore this property of TC might not have favored the healing of burn wounds. Even, this factor may be explaining the reason for not producing significant effect on the closure of burn wounds in standard group.

Lipid peroxidation is a common step in several types of injuries like burn, inflicted wound and skin ulcers for generating free radicals. 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. Any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils, increasing the strength of collagen fibers by an increase in vascularity [19], preventing the cell damage and promoting the DNA synthesis [20]. It is reported that antioxidants, such as vitamin C and vitamin E

have been shown to promote wound contraction and epithelization [21]. The phytochemical screening of TC revealed the presence of flavonoids [3] that are responsible for scavenging the free radicals [22] and diminution in the lipid peroxidation [23]. 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 [19] has prompted the healing of burn wounds.

CONCLUSION

It was observed from the results of the present study that the burn wounds treated with 2% TC cream promoted the healing of burn wound and also showed better wound healing activity as compared to the wound healing activity of the control and standard groups. TC promoted wound contraction and hastened the epithelization time 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. However, further studies should be carried out with isolated constituent of the extract to exactly determine the lead molecule responsible for the wound healing property.

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REFERENCES

1. Bingham HG, Hudson D, Popp J. A retrospective review of the burn intensive care admissions for a year. J Burn Care Rehabil 1995; 16:56-8.
2. McGill V, Kowal-Vern A, Fisher SG, Kahn S, Gamelli RL. The impact of substance use on mortality and morbidity from thermal injury. J Trauma 1995; 38:931-4.
3. Kirti sinha, Mishra NP, Singh J. Tinospora cordifolia (Guduchi), a reservoir plant for therapeutic applications: A review. J Indian traditional knowledge 2004; 3(3):257-270
4. Thatte UM, Kulkarni MR, Dahanukar SA. Immunotherapeutic modification of Escherichia coli peritonitis and bacteremia by Tinospora cordifolia. J Postgraduate medicine 1992; 38(1): 13-15
5. Ramya Premanath, Lakshmidevi.N. Studies on Anti-oxidant activity of Tinospora cordifolia (Miers.) Leaves using in vitro models. J American Science 2010; 6(10): 736-743
6. Rajalakshmi M, Eliza J, Cecilia Edel Priya, et al. Anti-diabetic properties of Tinospora cordifolia stem extracts on streptozotocin-induced diabetic rats. J African Pharmacy and Pharmacology 2009; 3(5):171-180
7. Biswadev B, Subhashree R, Soumya G and Mahuya S. Hepatoprotective and immunomodulatory properties of Tinospora cordifolia in ccl4 intoxicated mature albino rats. J Toxicological Sciences 2002; 27(3):139-146
8. Devprakash dahiya, K. K. Srinivasan, T. Subburaju, Sachin Kumar Singh. Antimicrobial Activity of Alcoholic and Aqueous Extracts of Tinospora cordifolia. J Pharmaceutical Science 2011; 2(1)
9. Fatima rose, Noorulla K M, Asma M, Vadivel K et al. Invitro antibacterial activity of methanolic root extract of tinospora cordifolia (willd). J International Pharma Research Development 2010; 2(5) :1-5
10. Bairy KL, Somayaji SN, Rao CM. An experimental model to produce partial thickness burn wound. Indian J Exp Biol 1997;35:70-2

11. Midwood, K.S.; Williams, L.V.; Schwarzbauer, J.E. Tissue repair and the dynamics of the extracellular matrix. J of Inter Biochemistry & Cell Biology 2004; 36 (6): 1031–1037.
12. Gabbaiani G, Harschel BJ, Ryan GB. Granulation tissue as a contractile organ. J Exp Med 1976; 135: 719.
13. Girish M, Kamdod M A. Effect of topical tinospora cordifolia on excisional wound in albino rats. Int J Pharmacy Biological Sciences 2012; 2(2):25-29
14. Girish M, Priyadarshini K. The influence of tinospora cordifolia on wound healing in albino rats. Int J Pharma Biosciences 2012; 3(2):379-384
15. Sharma A K and Singh R H, Screening of antiinflammatory activity of certain indigenous drugs on carrageenan induced hind paw oedema in rats. Bull medico Ethnobot Res 1980; 1(2): 12
16. Mary NK, Babu BH, Padikkala J. The antiatherogenic effect of an herbal formulation, Caps HT2, was evaluated as antioxidant, anticoagulant, platelet antiaggregatory, lipoprotein lipase releasing, anti-inflammatory and hypolipidaemic activity in rats. Phytomedicine. 2003; 10(6-7):474-82.
17. Rijswik L, Harding K, Bacilious N. Issues and clinical implications. Ostomy Wound Manage 2000; 46:51S-62S.
18. Faoagali J. Use of antiseptics in managing difficult wounds. Prim Intention 1999; 7:156-60.
19. Lawrence J. Coppey, Jill S. Gellett, Eric P. Davidson et al. Effect of Antioxidant Treatment of Streptozotocin-Induced Diabetic Rats on Endoneurial Blood Flow, Motor Nerve Conduction Velocity, and Vascular Reactivity of Epineurial Arterioles of the Sciatic Nerve. Diabetes 2001; 50, 1927-1937
20. Getie M, Gebre Mariam T, Reitz R, Neubert RH. Evaluation of the release profiles of flavonoids from topical formulations of the crude extract of the leaves of Dodonea viscosa (Sapindaceae). Pharmazie 2002; 57:320-2.
21. Rao CM, Ghosh A. Does metronidazole reduce lipid peroxidation in burn injuries, Indian J Pharmacol 1997;29:30-2
22. Kandaswami C, Middleton E Jr. Free radical scavenging and antioxidant activity of plant flavonoids.Adv Exp Med Biol. 1994; 366:351-76.
23. Heijnen CG, Haenen GR, Oostveen RM, Stalpers EM, Bast A. Protection of flavonoids against lipid peroxidation. Free Radic Res. 2002 May; 36(5):575-81.