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# INFLUENCE OF TINOSPORA CORDIFOLIA ON WOUND HEALING IN ALBINO RATS

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## ABSTRACT

**Objective:** To investigate the effect of tinospora cordifolia (TC) on resutured incision and dead space wounds in albino rats.
**Materials and Methods:** Resutured incision and dead space wounds were inflicted in albino rats under light ether anesthesia with aseptic precautions. Treatment group received tinospora cordifolia and control animals received vehicle orally for a period of 10 days. On the 11th day, after estimating breaking strength of resutured incision wounds, animals were sacrificed and granulation tissue removed from dead space wounds to estimate breaking strength, hydroxyproline content, quantification of granulation tissue and histopathological assessment in both control and treated groups.
**Results:** TC significantly ($P<0.05$) promoted the healing process in both the wound models studied.
**Conclusion:** TC promote wound healing, perioperative use of TC could promote the healing of surgical wounds.

## KEY WORDS

Tinospora cordifolia, Resutured incision wound, Dead space wound, Ether anesthesia

## INTRODUCTION

Wound healing, a common clinical entity contemporary to human beings often poses problems in clinical practice. Naturally, the investigative curiosity to promote healing continues since ages. Abundant research has been envisaged to develop better healing agents. Several drugs of plant origin like *Bryophyllum pinnatum* [1], *Moringa oleifera* [2], minerals like - zinc, iron and certain Vitamins like Vit-C, Vit-A are described for their wound healing properties in different wound models and were found to be effective. One such agent *Tinospora cordifolia* (TC) has not been subjected to scientific evaluation on wound healing.

'Tinospora cordifolia'(Guduchi or Amrita) [3] 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 [4], antioxidant [5], antidiabetic [6] and immunobiological [7] activities.

In view of the paucity of information of TC on wound healing the study was intended to explore the influence of TC on the process of wound healing. Hence, the aim of the present study was to investigate the influence of TC on resutured incision and dead space wounds in albino rats.

## MATERIALS & METHODS

***Animals and Drug treatment:***

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 of wounding site was performed a day before wounding. After wounding, the animals were divided into treatment and control groups (n=6 in each group) for each wound model. TC was obtained as generous gift from the Himalaya pharmaceutical company. The drugs were administered orally in therapeutic equivalent doses (per kg body weight) as calculated with the help of the conversion table devised by *'Paget and Barnes'* [8]. The dose of TC 200 mg/kg suspended in 2% gum acacia were administered once daily in the volume of 5mL/kg for the treatment group, whereas control group received equal volume of the vehicle. The duration of treatment was 10 days for both the experimental models studied (i.e. resutured incision and dead space wounds)

***Wound models***

1. Resutured incision wounds were inflicted with two 6 cm long paravertebral parallel incisions under light ether anesthesia as described by *Ehrlich HP, Hunt TK*. [9]. Sutures were removed on the 7th day. On the 10th post wounding day, breaking strength was measured by the continuous water flow technique as described by *'Lee'* [10] and subsequently animals were sacrificed by overdose of anesthesia.
2. Dead space wounds were inflicted and were implanted subcutaneously with sterile cotton pellets (10 mg) and cylindrical grass piths (2.5 cm × 0.3 cm) in the groin and axilla alternatively using the technique of *D'Arcy et al*. as described by *Turner* [11]. On the 10th post-wounding day, all the granulation tissues were removed under light ether anesthesia and dried at 60$^{o}$ C overnight to record the dry weight which was

expressed as mg per 100 g body weight as suggested by *'Dipasquale and Meli'* [12]. One of the granulation tissues over the grass piths was opened and trimmed to a rectangular piece for the estimation of breaking strength and subsequent colorimetric estimation of hydroxyproline content was performed as described by *Woessner JF* [13], whereas the other piece was preserved in 10% formalin for histopathological assessment.

***Statistical analysis***

The data were analyzed using Mann-Whitney U test. P values <0.05 were considered significant.

## RESULTS

The Significant promotion of wound-healing activity was observed with TC in both the wound models (resutured incision and dead space wound models) employed for the study.

In resutured incision wound model, TC treated animals showed significant increase ($p<0.005$) in mean breaking strength of wound (250.833± 27.823) as compared to that of controls (195 ± 11.401).

**Table.1**

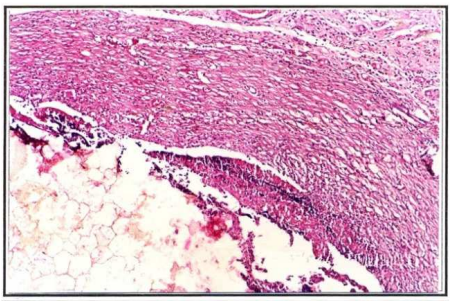
***Effect of Tinospora Cordifolia on resutured incision and dead space wounds (granulation tissue formation):***

| Sl.No/ (n=6) | Resutured wound Breaking Strength (g) | Dead space Wound (granulation tissue) | | |
|---|---|---|---|---|
| | | Breaking Strength (g) | Hydroxyproline Content (µg/g of tissue) | Dry weight (mg/100g) |
| **Control group -2% gum acacia (5ml/kg)** | 195 ± 11.401 | 307.50 ± 17.248 | 28.96 ± 2.067 | 46.63 ± 2.690 |
| **TC treated group (200mg/kg)** | 250.83± 27.823* | 369.16 ± 23.112* | 38.43 ± 2.888* | 54.55 ± 3.403* |
| **U -value** | 0.50 | 0.00 | 0.00 | 1.00 |
| **Z-value** | -2.8022 | -2.882 | -2.882 | -2.722 |
| ***P-value** | 0.0051 | 0.0040 | 0.0040 | 0.0065 |

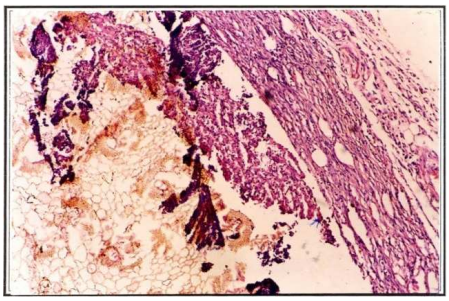
In dead space wound model, histological studies of the granulation tissue in the control group showed more aggregation of macrophages with scanty collagen fibres [Picture-2]. Whereas TC treated animal group evidenced significant increase in the number of fibroblasts and thick bundles of collagen tissue deposition with lesser macrophages [Picture-1]. Compared to the control group of animals, TC treated animals showed significant increase ($p<0.006$ and $p<0.004$) with respect to dry weight of granulation tissue (54.556 ± 3.4037) and breaking strength (369.166 ± 23.112) [Table-1]. Estimation of hydroxyproline content in the granulation tissue revealed that the animal groups treated with TC had high hydroxyproline content (38.436 ± 2.8888) as compared to that of control group (28.968 ± 2.0675). [Table-1]

**Microphotographs of Granulation Tissue Stained With H & E (100X)**

**Picture. 1**
**Tinospora Cordifolia:** Scanty granulation tissue with maximum fibrocollagen tissue



**Picture. 2**
**Control:** Abundant granulation tissue with scanty fibrocollagen tissue



# DISCUSSION

Results of the present study clearly indicate that TC in their therapeutic equivalent doses promotes healing of both the wound models employed in this study.

Wound healing is a fundamental response to tissue injury that results in restoration of tissue integrity, which is due to the synthesis of the connective tissue matrix. Collagen is a major protein of the extracellular matrix and is the component that ultimately contributes to wound strength [14]. Breakdown of collagen liberates free hydroxyproline and measurement of this hydroxyproline could be used as an indicator for collagen turnover [14]. The data depicted in [Table-1] reveal that the hydroxyproline content of the granulation tissue of animals treated with TC was significantly increased when compared to the control group, indicating increased collagen turnover. Increase in breaking strength of granulation tissue of TC treated animals indicate the enhanced collagen maturation by increased cross linking. In addition, increase in dry granulation tissue weight also indicated the presence of higher protein content [15]. This drug 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].

Probably by virtue of its antioxidant activity TC has reduced lipid peroxidation [16], which in-turn leads to prevention or delay in the onset of cell necrosis as well as improvement in the vascularity [17]. 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 [18]

Thus, wound-healing property of TC may be attributed to the phytoconstituents present in it, which may be either due to their individual or additive effect that hastens the process of wound healing. The component(s) of the extract that is responsible for this effect was not investigated as the study merely intended to explore the effect of TC on the process wound healing and not to probe its mechanism of action. Further phytochemical studies are required to identify and to isolate the active compound(s) responsible for these pharmacological activities.

# CONCLUSION

The peri-operative use of TC could promote healing of surgical wounds, if the present findings could be extrapolated to clinical situation, which necessitates further well planned clinical studies.

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## REFERENCES

1. Khan M, Patil PA, Shobha JC. Influence of Bryophyllum pinnatum (Lam.) leaf extract on wound healing in albino rats. J Natural Remedies 2004; 4:41-6. Back to cited text no. 1
2. Rathi B, Patil PA, Baheti AM. Evaluation of aqueous extract of pulp and seeds of Moringa oleifera for wound healing in albino rats. J Natural Remedies 2004; 4:145-9.
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. Paget GE, Barnes JM, Toxicity tests. In: Laurence DR Bacharach AL, editors. Evaluation of drug activities Pharmacometrics. London and New York: Academic Press; 1964.
9. Ehrlich HP, Hunt TK. The effects of cortisone and anabolic steroids on the tensile strength of healing wounds. Ann Surg 1969;170:203-6
10. Lee KH. Studies on the mechanism of action of salicylates II. Retardation of wound healing by aspirin. J Pharm Sci 1968; 57:1042-3.
11. Turner RA. 'Antiinflammatory agent' in screening methods of pharmacology. 2nd ed. New York: Academic Press; 1965.
12. Dipasquale G, Meli A. Effect of body weight changes on the formation of cotton pellet-induced granuloma. J Pharm Pharmacol 1965; 17:379-82.
13. Woessner JF. The determination of hydroxyproline in tissue samples. Arch Biochem 1961; 93:440.
14. Madden J W, Peacock EE. Studies on the biology of collagen during wound healing. 3. Dynamic metabolism of scar collagen and remodeling of dermal wounds. Ann Surg 1971; 174(3): 511–520
15. Azad S. Essentials of surgery. Hyderabad, India: PARAS Medical Publishers; 2002.
16. John M.C. Gutteridge. Lipid peroxidation and Antioxidants as Biomarkers of Tissue Damage. Clin.Chem 1995; 828 41/12, 1819-1
17. 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
18. 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.