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# Comparative Evaluation of the Wound healing efficacy of *Tinospora cordifolia* and local Insulin therapy in diabetic rabbits

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**ABSTRACT**
*Diabetes mellitus is one of the major contributors to delayed wound healing. So far there is no significantly effective therapy for diabetic wound healing. The present study aimed to evaluate the healing efficacy of Tinospora cordifolia and local insulin therapy in diabetic wounds. Eighteen animals were made diabetic using alloxan monohydrate. These animals were divided into two groups A and B each having nine animals. The wounds were created on the thoraco-lumbar area in each animal under general anesthesia. The animals of group A were treated with oral suspension prepared from methanolic extract of T. cordifolia stem and the animals of group B were treated with local insulin therapy. The soframycin ointment was applied over wounds in both the groups. The wound healing efficacy of T. cordifolia and local insulin therapy was assessed on the basis of wound condition, colour digital imaging, wound morphometery, histopathological and Scanning Electron Microscopic (SEM) studies. The rate of wound healing recorded in group B was significantly higher and better than group A. On the basis of observations of this study, it was concluded that T. cordifolia and local insulin therapy both enhanced the rate of healing in diabetic wounds but local insulin therapy shown the better healing efficacy.*
***Key words:*** *Diabetes mellitus, Wound healing, Tinospora cordifolia and Insulin.*

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## INTRODUCTION

Normal wound healing is a well orchestrated process that results in restoration of tissue integrity. This process is hampered in Diabetes mellitus leading to delayed wound healing. Persistence of inflammation and neutrophil infiltration are characteristics associated with impaired wound healing in diabetes [1,2]. Hyperglycemia is responsible for the development of infections in diabetic wounds. Bacteria thrive on the increased glucose available in the bloodstream and at the same time the high glucose concentration inhibits neutrophil action, leading to a proliferation of bacteria and infection. Infection and inflammation lead to imbalance of protease and reactive oxygen species, essential growth factors are degraded, the ability of angiogenesis is impaired and cell recruitment to the wound sites is also inhibited [3] consequently wound remains in a chronic inflammatory state. Management of such wounds poses a great challenge. Recently, there has been increasing interest in the use of medicinal plants because of their antidiabetic and wound healing properties. *Tinospora cordifolia* is one of them (Fig. 1a and 1b). It is a deciduous climbing shrub of the family *Menispermaceae.* It is distributed throughout the Indian subcontinent and China [4]. The plant has been reported as anti-diabetic and anti-hyperglycemic [5], anti-inflammatory [6], antioxidant [7], anti-stress [8] and wound healing properties [9]. In Diabetes mellitus there is absolute or relative lack of insulin which contributes to wound healing complications [10]. The role of local insulin in the promotion of wound healing has been reported by several workers [11,12,13]. It stimulates the growth and development of different cell types, and affects proliferation, migration and secretion by keratinocytes, endothelial cells, and fibroblasts [14]. It also stimulates protein turnover [15]

and promotes the synthesis of wound collagen, accelerates the wound angiogenesis and speeds up the wound healing process [16]. As, delayed wound healing is one of the most serious complications of diabetes mellitus and there are no significantly effective therapies for diabetic wounds so far. The Review of literature revealed the anti-diabetic and wound healing properties of *T. cordifolia* and Insulin. Hence, the present study was aimed to evaluate the wound healing efficacy of *Tinospora cordifolia* and local insulin therapy in diabetic animals.

## MATERIALS AND METHODS

### Preparation of methanolic extract of *Tinospora cordifolia*

To prepare the methanolic extract of matured stems of *T. cordifolia* (Fig-1c), the stems were chopped, shade dried and finely powdered. Soxhlet extractor was used to separate the extract from powder. The extract was dried and kept in refrigerator for further use. It was dissolved in distilled water @ 600 mg/2 ml. This prepared solution was given to the animals @ 2ml/ kg b.wt. orally, during the study period.

### Animals

Eighteen clinically healthy New Zealand white rabbits were used in this study. All the animals were kept separately in individual cages. Prior to start of this study, these animals were dewormed and kept under observation for one week to acclimatize the new environment and were randomly divided into two groups having nine animals each. The animals were fed green fodder, wheat grains, maize and grams, *ad libitum.* Fresh and wholesome water was also provided *ad libitum*. Similar conditions of management and feeding were continued to all the animals during the entire period of study.

### Induction of diabetes

For the induction diabetes, eighteen animals of both groups (9 animals in each) were made diabetic by administering the sterile solution of 5% alloxan monohydrate (Alloxan monohydrate –Titan Biotech Ltd. Bhiwadi, Rajasthan, India) in normal saline at the dose rate of 100 mg/kg body weight, intra-peritoneally as per the method described by Wang *et al.* [17]. To avoid the mortality due to hypoglycemia alloxan was administered in well fed rabbits after estimation of the normal serum glucose levels. The animals were given 2 grams of glucose/kg b.wt. dissolved in 10 ml of distilled water to counteract the anticipated alloxan induced hypoglycemia. The blood glucose levels were estimated on day zero, seven, fourteen, and twenty eight after alloxan administration by using blood glucose determination kit and rabbits having 300 mg/dl or more were considered as diabetic [18,19].

### Wound creation and design of study

Thoracolumbar area of the animals was prepared for strict aseptic surgery. The animals were anesthetized by administration of xylazine @ 10 mg/kg b.wt. and ketamine @ 50 mg/kg b.wt. intramuscularly [20]. After achieving surgical anesthesia, the animals were retained in ventral recumbency. The skin of thoraco-lumbar area was marked by sterile disk of 2 cm diameter to create the wounds of equal sizes in all animals. The skin was excised in full thickness. The wounds were treated as follow-

**Group A:** Administration of methanolic extract of *T. cordifolia* @ 2 ml/kg, per oral and antiseptic dressing of the wounds with soframycin ointment.

**Group B:** Administration of insulin locally @ 80 I.U. at the periphery of wound on alternative days and antiseptic dressing of induced wound with soframycin ointment.

### Assessment of wound healing

The wound healing efficacy of *T. cordifolia* and local insulin therapy was assessed on the basis of wound condition, color digital imaging, wound morphometery, histopathological and Scanning Electron Microscopic (SEM) studies.

### Wound condition

Wound condition was evaluated based on clinical parameters (swelling and exudation) as per method reported by Gangwar [21].

### Color digital imaging

Color digital photographs were taken on days 7, 14 and 28 with the help of digital camera at a fixed distance. Analysis of shape, size and irregularity of the lesion was determined.

### Wound morphometery

To calculate the percent contraction, the wound area of both the groups was measured as per method given by Bohling *et al.* [22] on postoperative days 7, 14 and 28.

### Histopathological study

Histopathological samples from the healing sites were collected on $7^{th}$, $14^{th}$ and $28^{th}$ day and preserved in 10% formal saline solution. After fixation of samples they were washed and dehydrated with a sequence of ethanol-xylene series of solutions [23]. The tissues were processed by routine paraffin ($54^0$C- $56^0$C

melting point) embedding technique and sections of 4-5 µ thickness were cut. The tissues were processed in a routine manner [24] and 4µ thick longitudinal sections were cut and stained with Hematoxylin and Eosin (H&E) as per the standard procedure [25].

**Scanning Electron Microscopic (SEM) Study**

For Scanning Electron Microscopic study, the wound specimens were collected from the test wounds on days fourteen and twenty eight, postoperatively. Each specimen consisted of both wound healing and adjacent soft tissue. The samples were fixed in 2% solution of glutaraldehyde in PBS (Cornovosky's fluid) for 24 hours. Further tissue processing was done as described by Gangwar [21].

**Statistical analysis**

SPSS 20 software was used for the statistical analysis of the collected data. The data was subjected to analysis of variance (ANOVA) and means were compared using Duncan's Multiple Range Test.

**RESULT AND DISCUSSION**

Wound condition was evaluated based on swelling and exudation from the wound. The mean values of swelling and exudation scores have been presented in Table-1. On 7$^{th}$ day, the extent of swelling was less in group B as compared to group A. On 14$^{th}$ day also the extent of swelling was lesser in group B as compared to group A. On 28$^{th}$ day, the swelling was almost absent in group B. But in group A still minute swelling was present. There was no significant difference between group A and group B from day 7 to day 28. On 7$^{th}$ day, the extent of exudation was less in group B as compared to group A. On 14$^{th}$ day, by this time the extent of exudation was very less in both the groups. It was minimum in group B followed by group A. On 28$^{th}$ day, in group A and group B exudation was inappreciable. But there was no significant difference in both the groups from day 7 to day 28.


**Fig. 1: a. *Tinospora cordifolia* b. Closer view c. Chopped mature stems**

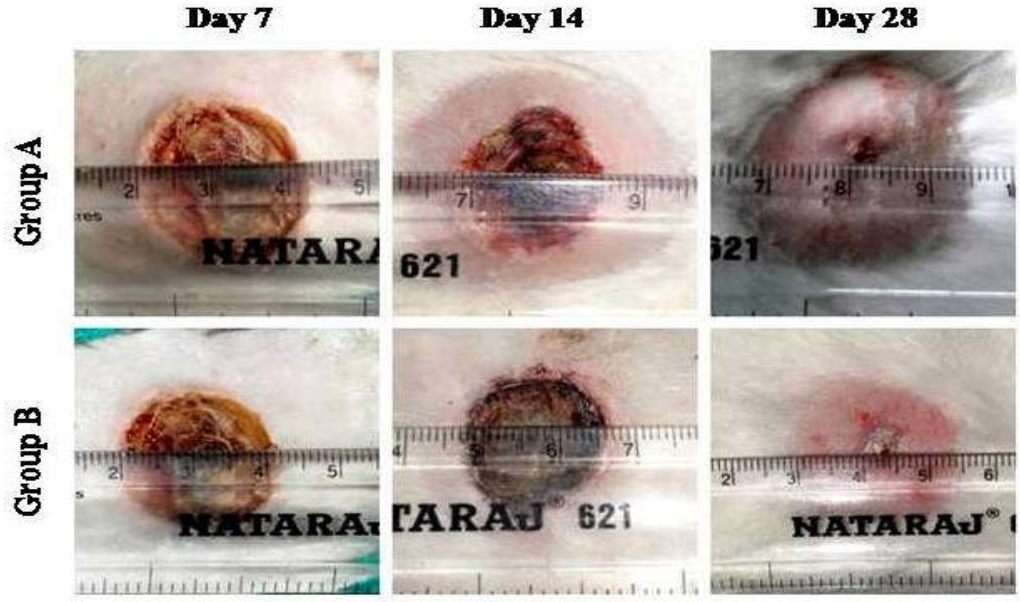


**Fig. 2: The colour digital photographs of the wounds of group A and B at different time intervals.**

The colour digital photographs of diabetic wounds treated with different therapies at different time intervals are presented in Figure 2. On days 7, 14 and 28, the wounds of the group B showed better healing than the group A. Wound contraction was also evident and better in group B when compared to group A. The percent of wound contraction was calculated by measuring the changes in wound size (Table- 2). On 7th day, the maximum wound contraction was recorded in animals of group B followed by group A. But there was no significant difference between both the groups at this stage. On 14th day, the wound contraction was still higher in group B while group A showed less contraction. On 28th day, the wounds were nearly healed in group B except in group A where only 79.71% wound contraction was recorded as compared to 94.68 % in group B. There was significant difference in wound contraction between group A and group B.

Histopathological evaluation of the tissue biopsy samples stained with H&E stain was done on different days (Fig. 3). On 7th day, In group A, there was very thin and incomplete layer of epithelium along with highly immature capillaries and granulation tissue, tissue debirs was having polymorphonuclear cells. In group B, there was incomplete but comparatively thick epithelium along with abundant highly vascular granular tissue as compared to group A but epidermis was not completely formed. Fibroblasts were present in abundant amount. On 14th day, in group A, superficial epithelium was not formed completely. Epidermal layer was not properly formed. Presence of newly formed capillaries (angeogenesis) and fibrosis in dermal tissue was evident at this stage. In group B, there was formation of epithelium along with incomplete epidermis. There was increase in connective tissue with evidence of collagen and newly formed blood vessels. On 28th day, in group A, epidermis was not properly formed, mild tissue reactions with few fibrous and collagen tissue were evident. In group B, epidermis was relatively formed and dermal area was filled with fibrous tissue.

Scanning Electron Microscopic study of the samples taken from wounds was also done at day 14 and 28 (Fig. 4). On 14th day, in group A, irregularly arranged collagenous tissue along with numerous newly formed blood vessels were seen. Epithelialization was in initial phase. In group B, irregularly arranged dense collagen tissue was seen with few blood vessels and epithelialization had started at this stage. On 28th day, in group A, the collagenous tissue became denser and arranged in a little bit regular pattern. The epithelial layer formed but was still incomplete and angiogenesis was reduced but still present. In group B, regularly arranged dense collagenous tissue was evident. The epithelial layers were formed angiogenesis was not evident at this stage.

In group A, swelling and exudation reduced slowly by 14th day after treatment. This might be due to the anti-inflammatory property of *T. cordifolia*. Rawal *et al.* [26] reported that *T. cordifolia* significantly enhances vascular endothelial growth factor (VEGF) and markedly decreases the expression of pro-inflammatory mediators such as *cyclooxygenase-2 (*COX-2) and cell adhesion molecules such as vascular cell adhesion molecule (VCAM). Singh *et al.* [27] reported that the anti-inflammatory activity of *T. cordifolia* might be due to berberine, cordifolioside A, cordioside, ecdysterone, isocolumbin, jatorrhizin, magnoflorine, palmatine and syringin. In group B (insulin treated group), the reduction in swelling and exudation may be due to the anti-inflammatory effect of insulin [28]. Insulin suppresses the inflammatory process, not only through preventing hyperglycemia but also by modulating key inflammatory molecules [29]. It inhibits nuclear factor-κB, activator protein-1 and early growth response-1 (EGR-1) which are major pro-inflammatory transcription factors. These factors regulate the expression of regulating monocyte chemotactic protein 1, intercellular adhesion molecule-1, matrix metalloproteinase (MMP)-2, MMP-9, tissue factor and plasminogen activator inhibitor-1, which also inhibited by insulin [30,31]. These proteins are important components of NADPH oxidase, which produces superoxide radicals with potent oxidative effects [32,33] leading to the damage of the tissue cells.

On day twenty eight, the mean wound contraction in group A was 79.71%. These results were in accordance with the findings of Barua *et al.* [34]. Closure of the *T. cordifolia* treated wounds might be associated with the increased keratinocytes proliferation and migration on the wound surface [3]. *T. cordifolia* also increases collagen turnover and collagen maturation by increased cross linking in wounds [35]. Inhibition of lipid peroxidation is 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 [36]. The antioxidant activity *T. cordifolia* reduces lipid peroxidation [37] which in-turn leads to prevention or delay in the onset of cell necrosis as well as improvement in the vascularity [38] leading to reduction in time of healing in diabetic wounds. Over all healing response observed in animals treated with oral suspension prepared from methanolic extract of stem powder of *Tinospora cordifolia* may be attribute to its antimicrobial [39], antioxidant [40], anti-inflammatory [41,42], immunobiological [43], antidiabetic [44] activities.

Significantly higher wound healing was recorded in group B animals when compared to group A animals. The wound healing activity of insulin may be attributed to its potent hypoglycemic activity and its ability to stimulate the growth and development of different cell types and effects proliferation, migration, secretion by keratinocytes, endothelial cells and fibroblasts [14,45,46,47,48]. Wu *et al*. [16] reported that topical or local application of insulin promoted the synthesis of wound collagen, accelerated the wound angiogenesis and wound healing by shortening the time needed for complete epithilialization in diabetic rats [49].

Furthermore, *T. cordifolia* is widely used as an herbal anti-hyperglycemic agent. However, it has an anti-hyperglycemic efficacy of 40% to 80% when compared to insulin [50]. This might also be an important factor for better wound healing in group B as control of hyperglycemia is of utmost importance to discourage the infections and hasten the diabetic wound healing.

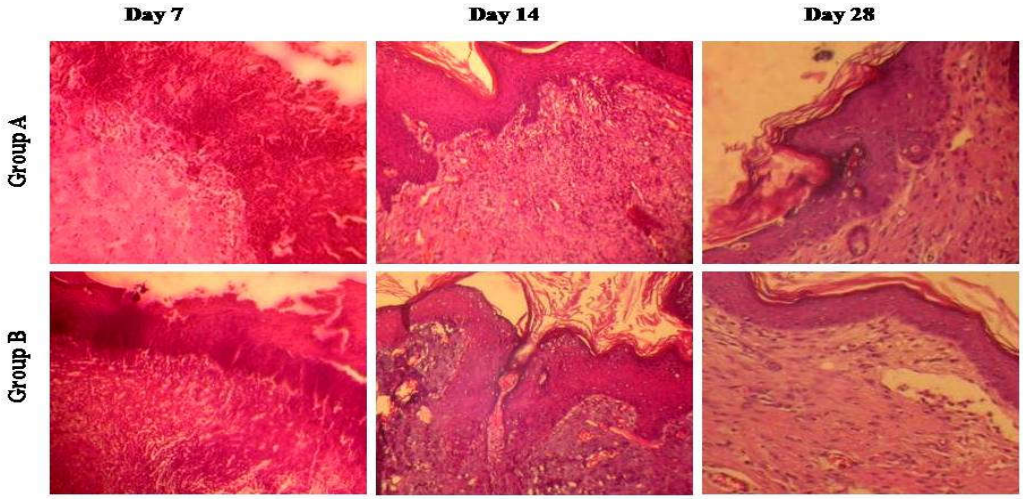


**Fig. 3: Histopathological observations of the wound biopsies collected from the animals of groups A and B at different time intervals (H&E, 40x).**

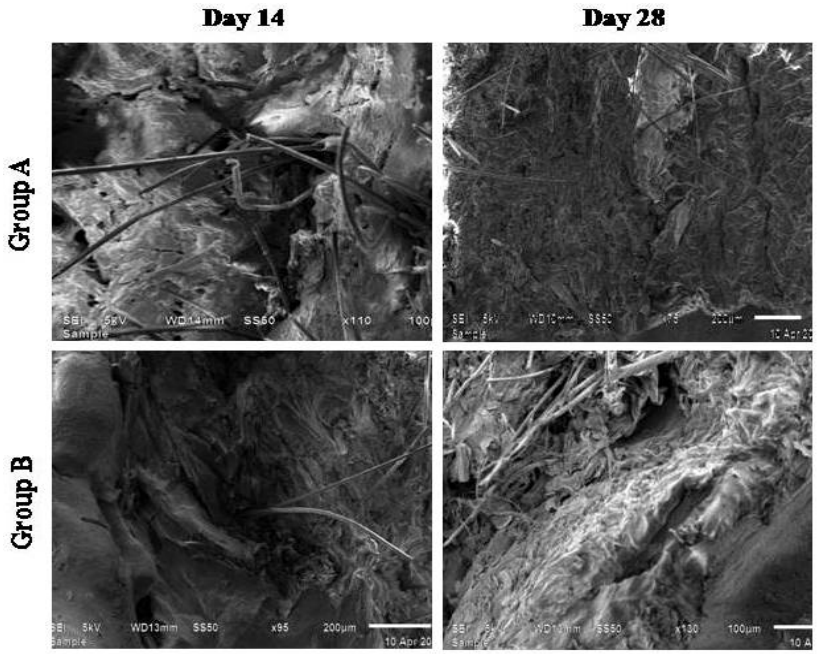


**Fig. 4: Scanning Electron Microscopic (SEM) pictures of the healing sites in group A and B at different time intervals.**

**Table-1: Mean values of swelling and exudation scores of group A and B at different time intervals**

| S.N. | Clinical parameters | Days | A | B |
|---|---|---|---|---|
| **1.** | **Swelling** | 7 | 2.66 ±0.33 | 2.33 ±0.33 |
| | | 14 | 2.00 ±0.58 | 1.33 ±0.33 |
| | | 28 | 0.33 ±0.33 | 0.33 ±0.33 |
| **2.** | **Exudation** | 7 | 2.66 ±0.33 | 2.00 ±0.58 |
| | | 14 | 1.33 ±0.67 | 0.66 ±0.33 |
| | | 28 | 0.33 ±0.33 | 0.33 ±0.33 |

**Table-2: Mean wound contraction (%) in group A and B at different time intervals**

| S.N. | Groups | Days | | |
|---|---|---|---|---|
| | | **7** | **14** | **28** |
| **1.** | **A** | 12.94 ±3.07 | 27.59 ±4.96 | 79.71 ±2.58 |
| **2.** | **B** | 21.75 ±7.74 | 43.83 ±4.32 | 94.68 ±0.74 |

## CONCLUSION

Delayed wound healing is one of the major complications of Diabetes mellitus which is due to hyperglycemic conditions of wound. *Tinospora cordifolia* and insulin both have been reported to have anti-diabetic, anti-iflammatory and wound healing properties. Therefore, in the present study healing efficacy of *Tinospora cordifolia* and local insulin therapy in diabetic wounds was evaluated. On the basis of observations of this study, it was concluded that oral administration of methanolic extract of *T. cordifolia* and local insulin therapy both have enhanced the rate of wound healing in diabetic animals however the local insulin therapy has shown the better efficacy.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

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