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Evaluation of Wound healing activity of *Tinospora cordifolia* Willd.

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ABSTRACT

The methanolic extract of roots of Tinospora cordifolia was screened for the wound healing activity in the form of ointment on albino mice using two types of wound models viz, excision, incision. The parameters studied include percentage of wound contraction, period of complete epithelialization and tensile strength of incision wound and histological studies of granulation tissue. The extract ointments showed considerable difference in response in the above said wound models as comparable to those of a standard drug (Povidone iodine ointment) and control (simple ointment base). Histopathological study of the granulation tissue of methanoicl extract treated animals showed few monocytes with increase in collagenation when compared to control group of animals. The results revealed that methanolic extract of roots of Tinospora cordifolia have significant ($p< 0.01$) wound healing activity as compared to control.

Key words: Excision wound model, Incision wound model, Povidone iodine, Wound healing.

INTRODUCTION

Wounds are the physical injuries that result in an opening or breaking of the skin and appropriate method for healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin [1]. *Tinospora cordifolia* (Willd) Miers (Menispermaceae) also called Guduchi is an herbaceous vine indigenous to the tropical areas of India, Myanmar and Sri Lanka. *Tinospora cordifolia* Miers is a plant prescribed in Ayurveda, the Indian traditional system of Medicine as a 'Rasayana' or general tonic [2]. The active principles of *Tinospora cordifolia*, a traditional Indian medicinal plant were found to possess anticomplementary and immunomodulatory activities [3, 4]. It is listed as an insecticide, an antifungal agent, an antibacterial agent and a fish toxin [5]. Dry barks of *T. cordifolia* has antispasmodic, anti-pyretic [6], anti-allergic [7] anti-inflammatory [8] and anti-leprotic [9] properties. Guduchi is a promising drug entity which should enter the world market by evidence-based research for therapeutics [10]. It is also considered to be analgesic, aphrodisiac, diaphoretic, emetic, emmenagogue, stimulant and tonic [11]. A survey of literature revealed that the roots of *Tinospora cordifolia* has not been scientifically investigated for its wound healing activity. Thus on traditional use & and literature references, methanolic root extract of *Tinospora cordifolia* is evaluated for its wound healing activity.

MATERIALS AND METHODS

Plant material

The sample was collected from the Narsingpur region of Madhya Pradesh in the month of October. The plant was selected as *Tinospora cardifolia* on basis of morphological charachterstics and authenticated in the Department

of Botany, Saifia College of Science and Education, Bhopal by Dr. Zea Ul Hasan. The Reference no. is Bot. 117, 2/12/2010. A voucher specimen has been deposited in our laboratory for further reference.

Preparation of extract

The roots of *Tinospora cordifolia* were air dried in the shade with the precaution of contamination from the dust. It was then grinded with the help of the simple hand grinder. It was crushed into fine powder, and stored into the air tight jars preventing from moisture, and kept for further analysis. Powdered root (100 gm) was extracted in soxhlet apparatus for 24 hours with methanol then concentrated and dried. The extract was weighed and percentage yield was calculated (18.44% w/w).

Preparation of formulation

The 10% ointment formulation were prepared for topical administration, 10 g each of the methanolic extract of *Tinospora cordifolia* roots were separately incorporated with 100 g of Petroleum jelly B.P. The formulation was stored in well tight container and kept in cool and dry place.

Experimental Animal

Albino mice of either sex (weighing 35 to 40 gms) were selected for wound healing activity. Mice were divided into three groups, each group having six animals. The study was conducted after obtaining the approval of the Institutional Animal Ethics Committee (Reg. No. IAEC/BITS-P/004). Group I was assigned as control received simple ointment base I.P. Group II received the standard drug (5% w/w povidone-iodine ointment and Group III received ointment of methanolic extract of roots of *Tinospora cordifolia*.

Excision wound

Excision wound was inflicted by cutting away circular piece (approximately 10 mm) of full thickness of shaved skin of a predetermined area on the anterior-dorsal side of each mice. The extract ointments were applied once daily to treat (group III) animals. The simple ointment base and povidone iodine ointment were applied in the same way to serve as control (group I) and standard (group II) respectively. The measurement of wound area were was done by using verniar calipers on 4^{th} , 8^{th} and 12^{th} post wounding day till the wound was completely healed [12]. The % of wound contraction (wound healing) area was calculated by using following formula:

$$\text{Percentage of Wound contraction} = \frac{\text{Initial wound size} - \text{specific day wound size}}{\text{Initial wound size}} \times 100$$

Incision wound

In the incision model [13], the mice were general anaesthetized by anesthetic ether and longitudinal paravertebral incisions of 1 cm length were made through the entire length of skin. After the incision was made, the part of skin was kept together and stitched with surgical thread (No. 000), 1 cm apart, using a curved needle (Needle no. 11). The wound were left undressed. The drug were given by topical route once a day, till complete healing The sutures were removed on the 8th day and complete healing (tensile) strength was measured on the 10th day of healing of incision wound. The tensile strength was measured by the method of hanging water bottle method [14]. The data obtained were calculated for the mean and the standard deviation. The student t test was applied over the readings. The observation were given in the table 2.

Histopathological study

It was performed by cutting the thin sections of the experimental animal skin on the 12^{th} post wounded days. For histological studies, granulation tissues were fixed in 10% neutral formalin solution for 24 h and dehydrated with a sequence of ethanol-xylene series of solutions. The materials were infiltered and embedded with paraffin (40-60 °C). Microtome sections were taken at 10 µ thickness. The sections were processed in alcohol-xylene series and stained with hemotoxylin-eosin dye [15]. After complete staining the slides, microscopic photographs of collagen tissue were taken as were shown in the figure (1, 2, 3) for control, standard and treated group.

RESULTS AND DISCUSSION

In the excision wound model, the test formulation treated animals showed high percentage closure of wound area which is more or less similar to the values of standard drug treated group where as the rate of wound contraction was

less in control group of animals, indicating the effect of the plant on promoting healing of excision wound. The results of excision wound studies are shown in table1.

Table 1: Percentage Wound Contraction Area Left of Excision Wound Area on the following Post Wounding Days.

Percentage wound contraction area (mm^2) (mean)			
Days	**Control Simple ointment Base**	**Standard (Povidone iodine)**	**Treated (Ointment containing 10% Root Extract**
4^{th}	31.13±2.32	72.72±0.68	52.54±7.46
8^{th}	63.1±0.346	99.38±0.72	85.08±1.56
12^{th}	73.22.01±0.788	99.9±0.02*	99.07±0.622*

*Each value is a mean percentage closure area ± Standard Deviation for group of six animals (n=6). *P< 0.01 indicates significant when compared to control group.*

Table 2: Mean tensile strength (gm) of incision wound on 10^{th} post wounding day.

Day	Tensile strength gm±sd		
	Control	**Standard**	**Treated**
10^{th}	179.62±2.71	426.4±0.94	399.43±1.78*

*Each value are in mean ± standard deviation for the group of six animal (n=6). *p<0.01*

CONTROL GROUP

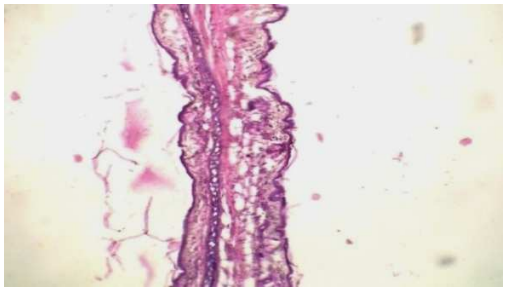

Figure1: shows less collagen formation which is not significant and incomplete epidermis in the 12^{th} post wounded day of the control group of exision wound model. H & E Stain, Magnification 10x.

STANDARD GROUP

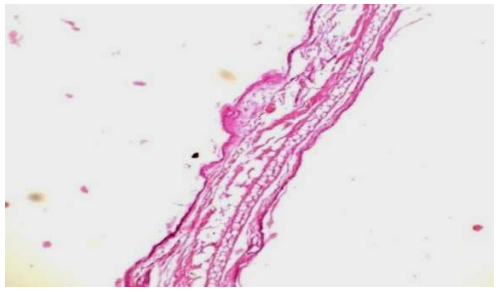

Figure2: shows enhance collagen formation and complete epidermis and less infiltration monocytes which received the treatment of Standard drug. H & E Stain, Magnification 10x

TREATED GROUP

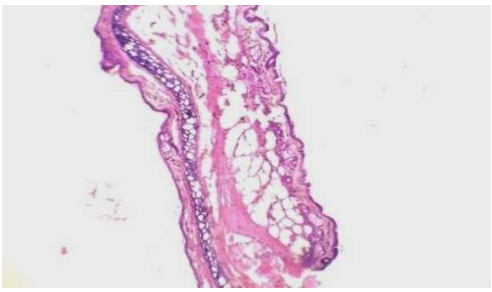

Figure3: shows enhance collagen formation and complete epidermis which received the treatment of *Tinospora cordifolia* extract ointment. H & E Stain, Magnification 10x

In incision wound model, the incision wounds treated with test formulation (methanolic root extract ointment) and standard (povidone iodine) show tensile strength 399.43±1.78 g and 426.4±0.94 g, respectively, compared with tensile strength of control group, i.e., 179.62±2.71 g The above observation for tensile strength shows that the incision wounds treated with extract formulation show significant increase in the tensile strength as compared to control group indicating the effect of *Tinospora cordifolia* root extract in maturation of collagen fibres. This shows wound healing potential of tested formulation. The results are mentioned in table 2.

The histopathological results of test formulation were compared with the results of control group (negative control) and results of standard povidone iodine (positive control). Histological studies of granulation tissue of the control group showed aggregation of more number of macrophages and less collagen fibres. In methanoicl extract treated animals, significant increase in collagen deposition with lesser macrophages was noticed.

CONCLUSION

In the present study, the phytochemical investigations of root extracts showed the presence of triterpenoids, saponins, alkaloids and flavonoids. Several phytoconstituents like tannins [16], flavonoids [17], triterpenoids [18] and sesquiterpenes [19] are also known to promote the wound-healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for wound contraction and increased rate of epithelialisation. Flavonoids are known to reduce lipid peroxidation and any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils by increasing the strength of collagen fibres, increasing the circulation, preventing the cell damage and by promoting the DNA synthesis [20]. The sesquiterpene lactones are known to possess antioxidant property [21, 22], which may also contribute to the wound-healing process. The present study revealed that methanolic extract possesses significant wound healing promoting activity. The present study revealed that the methanolic extract of *Tinospora cordifolia* possesses better wound healing potency, which was evident by the increased rate of wound contraction; reduction in the period of epithelialization, increase in collagen deposition and increase in tensile strength in granulation tissue.

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