WOUND HEALING ACTIVITY OF HYDROALCOHOLIC EXTRACTS OF *TYLOPHORA INDICA* LEAVES IN RATS

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Summary

The seeds of *Tylophora indica* are used traditionally in the folklore for the treatment of various kinds of wounds. The present study was undertaken to verify the effect of *Tylophora indica* leaves on experimentally induced wounds in rats in excision wound, incision wound, burn wound and dead space wound models. *Aloe vera* was used as standard wound healing agent. A formulation of hydroalcoholic extract *Tylophora indica* (HETI) was prepared in emulsifying ointment at a concentration of 5% & 10% and applied to the wounds. In the excision wound and burn wound models, where the so treated animals showed significant reduction in period of epithelization and wound contraction -50%. In the incision wound model, a significant increase in the breaking strength was observed. *Tylophora indica* treatment orally (100 mg and 250 mg/kg) in dead space wound model, produced a significant increase in the breaking strength, dry weight and hydroxyproline content of the granulation tissue in the dead space wound. The results suggest that *Tylophora indica* leaves extract applied topically or administered orally possesses wound healing activity.

Keywords: Hydroalcoholic extract of *Tylophora indica*, incision wound, excision wound, dead space wound, burn wound.

Introduction

Wounds are inescapable events of life and they arise due to physical trauma, chemical injury or microbial infections. Healing of wounds usually takes place in a direction away from its normal course and under-healing, over-healing or no healing of wounds is common. Management of under healing of wounds is a complicated and expensive program and research on drugs that increase wound healing is a developing area in modern biomedical sciences. Several drugs obtained from plant sources are known to increase the healing of different types of wounds. Some of these drugs have been screened scientifically for evaluation of their wound healing activity in different pharmacological models and patients, but the potential of many of the traditionally used herbal agents remains unexplored. In few cases, active chemical constituents were identified (1).

Epidemiologic studies show an inverse correlation between herbal therapies such as *Tylophora indica* and progression of wound. The leaves of this plant are traditionally used as a folk remedy in certain regions of India for the treatment of bronchial asthma and bronchitis (2). The extract of tylophora is known to have anti-oxidant and potent NO scavenging activity (3). The leaves and roots of *Tylophora indica* were traditionally used as anti-inflammatory, antiallergic and wound healing remedy (4, 5). However, there is no scientific report for confirmation of their wound healing activity. Thus, the present study was undertaken to ascertain the effect of hydroalcoholic extract of *Tylophora indica* leaves (HETI) on experimentally induced wounds in rats.

Methods

Experimental animals

Male albino Wistar rats weighing between 250-275 g were used. The animals were caged individually after wounding for treatment till completion of wound healing. The experimental protocol was approved by Institutional Animal Ethics Committee and animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Chemicals

Ketamine injection was procured from Prem Pharmaceuticals Pvt. Ltd. (Indore, India) and xylazine was from Indian Immunological Ltd. (Guntur, India), hydroxyproline and paradimethylamino benzaldehyde were procured from SD Fine Chemicals Pvt. Ltd. (Mumbai, India), All other chemicals used were also of analytical grade and purchased from standard companies.

***Plant extraction,* Selection of dose, gel base and treatment period**

The leaves of *Tylophora indica* was purchased from medicinal garden Danvantri vana, Bangalore University, in the month of June 2007. The plant material (Voucher Specimen No.-RRCBI 0691) was authenticated by Regional Research Institute (Ay.), Bangalore. The leaves were shade dried and powdered (moderately coarse). The extraction was carried out with 70% of methanol in soxhlet for about 72 hrs. The obtained syrupy mass was concentrated and dried in hot air oven. The doses for topical administration was used based on the information provided by the traditional healers and the oral dose was selected assuming that seeds are very safe at a dose of 5 g/kg, *p.o* as per limit tests of OECD guidelines (6), $1/50^{th}$ and $1/25^{th}$ of the safe dose corresponding to 100 mg/kg and 250 mg/kg were used for oral administration. The extract was formulated as 5% (w/w) and 10 % (w/w) emulsifying ointments (7). HETI 5% (w/w) in emulsifying base was used as a low dose and HETI 10% (w/w) was used as a high dose for topical application. For oral administration, suspension of HETI crushed sesame seed (100 mg/kg and 250 mg/kg) was prepared using acacia (5%) as suspending/emulsifying agent. *Aloe vera* extract (300 mg/kg, *p.o*) in the form of suspension (acacia 5%) was used as standard drug (8). The treatment period was 10 days for incision and dead space wound models and in case of excision and burn wound models, the treatment was continued till the day of scab falling.

Excision wound (9, 10)

The animals were anesthetized using ketamine (100 mg/kg, im) and xylazine (16 mg/kg, im). An impression was made on the dorsal thoracic region 1 cm away from vertebral column and 5 cm away from ear on the anaesthetized rat. The particular skin area was shaved one day prior to the experiment. The skin of impressed area was excised to the full thickness to obtain a wound area of about 500 mm^2. Haemostasis was achieved by blotting the wound with cotton swab soaked in normal saline. The animals were then grouped and different formulations were applied to cover the entire wounded area as follows: Group I: Control, Group II: *Aloe vera* extract (10%) gel formulation, Group III: HETI (5%), Group IV: HETI (10 %.). Wound area was measured by tracing the wound on a millimeter scale graph paper on predetermined days i.e., 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 days post-wounding. The wound contraction-50% (days) was determined by plotting the wound area Vs days on a graph paper. Falling of scab leaving no raw wound behind was taken as end point of complete epithelization and the days required for this was taken as period of epithelization.

Incision wound (11-13)

Para vertebral straight incision of 6 cm length was made through the entire thickness of the skin, on either side of the vertebral column with the help of a sharp scalpel. After complete haemostasis, the wounds were closed by means of interrupted sutures placed at approximately 1 cm apart. Animals were treated daily with drugs, as mentioned above under excision wound model from 0^{th} day to 9^{th} post-wounding day. The wound breaking strength was estimated on 10^{th} day by continuous, constant water flow technique.

Burn wound (14)

Partial thickness burn wounds were inflicted on overnight-starved animals under ketamine (100 mg/kg, im) and xylazine (16 mg/kg, im) anesthesia by pouring hot molten wax (2 g) at 80 ^{0}C. The wax was poured on the shaven back of the animal through a cylinder of 300 mm^2 circular opening. The wax was allowed to remain on the skin till it gets solidified. Immediately after the injury and on subsequent days, the drugs or base was applied topically as mentioned above.

Dead space wound model (15)

This type of wound was created by implanting subcutaneously a 2.5x0.5 cm polypropylene tube in the lumber region in anesthetized rats. Animals received one of the following treatments from 0^{th} day to 9^{th} post wounding day. Group I: 5% acacia solution (control), Group II: *Aloe vera* extract (300 mg/kg, po), Group III: HETI (100 mg/kg, po), Group IV: HETI (250 mg/kg, po). On the 10^{th} post wounding day, the animals were sacrificed and the granulation tissue harvested on the implanted tube was carefully dissected out along with the tube. The tubular granulation tissue was cut lengthwise to obtain a sheet of granulation tissue. The breaking strength was measured as described under incision wound model. The pieces of granulation tissue were collected, dried at 60 ^{o}C for 24 hr to get a constant weight and weighed. The tissue was then used for the determination of hydroxyproline content (16).

Statistical analysis

Results are expressed as mean ± SEM. The differences between experimental groups were compared using one-way Analysis of Variance (ANOVA) followed by Bonferroni's test. The results were considered statistically significant when $P<0.05$.

Results

Effect on excision and incision wound

Both high as well as low concentration of hydroalcoholic extract of *Tylophora indica* (HETI) produced a significant decrease in period of epithelization when compared to control ($P<0.01$). Treatment with Standard *Aloe vera* extract also produced significant reduction in the period of epithelization ($P<0.01$). All the treatments also showed significant decrease in wound contraction (50%) as compared to control ($P<0.001$). It was also found that the high dose (10 %) of HETI was comparatively more effective than low dose (5%) of HETI in reducing the epithelization period (Table 1).

The breaking strength of 10 days old incision wound was increased by all treatments. The high dose of HETI was more effective than low dose and standard in increasing the breaking strength of the incision wound (Table 1).

Table 1: Effect of hydroalcoholic extract of *Tylophora indica* (HETI) on the period of epithelization and wound contraction 50% in excision wound model and breaking strength in incision wound model

Groups	**Excision wound**		**Incision wound**
	Epithelization period (days)	**Wound Contraction-50% (days)**	**Breaking strength**
Control (Emulsifying base)	22.21 ±0.21	9.93±0.30	411.66±14.47
Standard *Aloe vera* extract (300 mg/kg, po)	17.63±0.23**	7.96±0.34**	463.33±11.94*
HETI (5%) (Emulsifying base)	18.41±0.21**	7.43±0.30**	518.33±11.37**
HETI (10%) (Emulsifying base)	14.91±0.40**	5.90±0.42**	641.66±11.94**

All values are mean± SEM, n=6, **P<0.01 vs. control.

Effect on burn wound

Like the excision wound model, application of HETI (5%), HETI (10%) as well as Standard *Aloe vera* extract (300 mg/kg, po) topically shortened the period of epithelization significantly ($P<0.001$) and also produced a significant decrease ($P<0.001$) in wound contraction-50% (days) when compared to control. The high dose of HETI was found to be more effective when compared to low dose of HETI and standard. (Table 2).

Table 2: Effect of hydroalcoholic extract of *Tylophora indica* (HETI) on the period of epithelization and wound contraction 50% in burn wound model.

Groups	**Burn wound**	
	Epithelization period (days)	**Wound Contraction-50% (days)**
Control (Emulsifying base)	21.16 ±0.30	11.33±0.33
Standard *Aloe vera* extract (300 mg/kg, po)	19.00±0.36**	9.16±0.16**
HETI (5%) (Emulsifying base)	18.10±0.16**	8.33±0.33**
HETI (10%) (Emulsifying base)	15.16±0.60**	6.16. ± 0.31**

All values are mean± SEM, n=6, **P<0.01 vs. control.

Effect on dead space wound

The breaking strength of 10 days old granulation tissue was significantly promoted by all the treatments; HETI (100 mg/kg, po), HETI (250 mg/kg, po) and *Aloe vera* extract (300 mg/kg, po). The dry tissue weight and hydroxyproline content were significantly increased ($P<0.001$) by all the treatments when compared to control. (Table 3).

Table 3: Effect of hydroalcoholic extract of *Tylophora indica* (HETI) on breaking strength, dry tissue weight and hydroxyproline content in dead space wound model.

Groups	**Breaking strength (g)**	**Dry tissue weight (g)**	**Concentration Of Hydroxyproline (µg/g of tissue)**
Control (Emulsifying base)	284.16 ± 8.983	69.16 ± 4.729	2533.33 ± 245.85
Standard *Aloe vera* extract (300 mg/kg, po)	535.83 ±12.001***	175.83 ± 7.574***	6266.66 ± 245.85***
HETI (100 mg/kg)	497.50 ± 8.732***	157.33 ± 5.232***	5466.66 ± 321.11***
HETI (250 mg/kg)	540.00 ± 2.845***	165.00 ± 5.859***	6266.66 ± 245.85***

All values are mean± SEM, n=6, **P<0.01 vs. control.

Discussion

The present study was undertaken to evaluate whether *Tylophora indica* leaves promote wound healing in experimentally induced wounds in rats. The results of the present study substantiate the use of *Tylophora indica* leaves in folklore medicine for the treatment of wounds. The emulsifying base containing hydroalcoholic extract applied topically promoted the breaking strength, wound contraction and period of epithelization in different models of experimental wounds.

Collagenation, wound contraction and epithelization are crucial phases of wound healing. The phases of inflammation, macrophasia, fibroplasia and collagenation are intimately interlinked. Thus an intervention into any one of these phases by drugs could eventually either promote or depress one, other or all phases of healing. Growth hormone is known to promote the healing process by enhancing epithelial cell proliferation and cell collagen formation. Collagen is the family of protein, which provide structural support and it is the main component of tissue such as fibrous tissue, cartilage. The collagen synthesis is stimulated by various growth factors (17). Growth hormone is also known to promote the proliferation of fibroblasts (18) and fibroblast proliferation form the granulation tissue.

In the dead space wound model, *Tylophora indica* treatment increased granuloma tissue weight and breaking strength. The exact mechanism(s) by which HETI increased the granuloma tissue weight and breaking strength of granulation tissue can not be explained with the present data.

Lipid peroxidation is an important process of several types of injuries like burn, inflicted wound and skin ulcers. A drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils, increasing the strength of collagen fibers by an increase in circulation, thereby preventing the cell damage and promoting the DNA synthesis (19). Several antioxidants such as vitamin C, metronidazole and vitamin E are reported to increase the wound healing (20). As seen in the present study as well as discussed elsewhere (3), extract of tylophora possess strong anti-oxidant and potent NO scavenging property by virtue of its flavonoids contents, hence it can be suggested that the wound healing activity of HETI after both local and systemic administration may at least be in part due to its potent antioxidant activity.

To conclude, leaves of *Tylophora indica* possess good wound healing activity when applied locally. The high dose of the extract was found to be more effective topically than the low dose. Further, isolation of active constituents from the extracts of the leaves may bring about the development of a new wound-healing agent.

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