ORIGINAL ARTICLE

EVALUATION OF *TECTONA GRANDIS* LEAVES FOR WOUND HEALING ACTIVITY

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ABSTRACT

The frontal leaves of *Tectona grandis* (Verabinaceae) are widely used in the folklore for the treatment of various kinds of wounds, especially burn wound. The present study was carried out to evaluate the effect of hydrochloric extract of *Tectona grandis* on experimentally induced wounds in rats and compare the effects observed with a known wound healing agent, *Aloe vera*. The models selected were excision wound, incision wound, burn wound and dead space wound. A suitable gel formulation was selected for the application using cellophane membrane penetration. In the excision wound and burn wound models, animals treated with *Tectona grandis* leaf extract showed significant reduction in period of epithelisation and wound contraction 50%. In the incision wound model, a significant increase in the breaking strength was observed. *Tectona grandis* leaf extract treatment orally produced a significant increase in the breaking strength, dry weight and hydroxyproline content of the granulation tissue in dead space wound. It was concluded that *Tectona grandis* leaf extract applied topically (5% and 10% gel formulation) or administered orally (250 mg and 500 mg/kg body weight) possesses wound healing activity.

Keywords: *Tectona grandis*; incision wound; excision wound; dead space wound; burn wound.

INTRODUCTION

Wounds are inescapable events of life; wound may arise due to physical, chemical or microbial agents and wound healing has been one of the earliest medical problems. Healing is essentially a survival mechanism and represents an attempt to maintain normal anatomical structure and function. Healing of wound takes place in a direction away from its normal course and it is common to have none, under or over healing. Treatment is therefore aimed at either shortening the time required for healing or minimizing the undesired consequences. Advances in surgical skill and technique have overcome the latter to some extent.

Management of wound healing, particularly the under healing is complicated and expensive programme. Research on wound healing drugs is a developing area in modern biomedical sciences. Several drugs from plant are known to have wound healing properties. Some of these plants have been screened scientifically for evaluation of their wound healing activity in different pharmacological models and patients, but the potential of most remains unexplored. In a few cases, active chemical constituents were identified (Biswas and Mukherjee, 2003). Hence, there is a dearth of safe, economic and effective pro-healing agents for the wound management programme, which can enhance healing as well as control infection.

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The plant, *Tectona grandis* (Oudhia, 2006) is extensively used in folklore for treatment of burn wound but no scientific data is available. The present work deals with the screening of *Tectona grandis* leaf extract for wound healing activity in rats.

Tectona grandis is commonly known as Indian teak, and it belongs to family Verabinaceae. Lapachol, a napthaquinone isolated from *Tectona grandis* is reported to have anti-ulcer (Goel *et al.*, 1987) and nitric oxide scavenging activity (Jagetia and Baliga, 2004).

MATERIALS AND METHODS

Experimental animals

Male albino Wister rats weighing between 250-275 gm were used in the present study. 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

Pentobarbitone sodium was obtained from Sigma-Aldrich (USA). Hydroxyproline and Paradimethylamino benzaldehyde were procured from S.D. Fine Chemicals Pvt Ltd. (Mumbai, India). Sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2) and copper sulphate ($CuSO_4$) were purchased from Nice Chemicals Pvt. Ltd. (Mumbai, India). Hydrochloric acid (HCl) was obtained from

Ranbaxy Fine Chemicals Pvt. Ltd (Mumbai, India). Hydroxypropyl methylcellulose (HPMC) and Carboxymethylcellulose (CMC) were purchased from Loba Chemie (Mumbai, India) and Na-Alginate was from Thomas Baker (Mumbai, India). *Aloe vera* (Standard drug) gel formulation was obtained from Abhi Natural Products Pvt. Ltd (Pune, India).

Extraction of Tectona grandis leaves

The frontal fresh leaves of *Tectona grandis* were collected from Koramangala area of Bangalore, India in the month of December. The Regional Research Institute, Bangalore identified the plant. A voucher specimen (RRCBI, ACC. No. 12474) has been preserved in the institute for future reference. The leaves were air dried for 12 days and powdered in hand grinder to get coarse powder. The powdered leaves were extracted for 30 hr in a Soxhlet apparatus using hydro-alcoholic mixture (70% methanol) as solvent. The hydro-alcoholic extract was vacuum dried followed by desiccation to get the constant weight. The *Tectona grandis* extract (TG) thus obtained was subjected to preliminary phytochemical analysis.

Selection of gel base

The extract was formulated as a gel using polymers such as hydroxypropylcellulose (HPMC)-7.5%, carboxy-methylcellulose (CMC) -7.5% and Na-alginate -7.5% to obtain an easily applicable topical system. Quantitative release of tannin from each of the formulation was evaluated using a semipermeable cellophane membrane (Pandey *et al.*, 1998).

Acute oral toxicity study

(http://www.epa.gov/oppts/home/guideline.htm)

The acute oral toxicity study was performed according to the OPPTS (Office of Prevention, Pesticides and Toxic Substance) guidelines following limit test procedure.

Selection of dose and treatment period

Tectona grandis (5%) in HPMC (7.5%) was used as low dose (TG 5%) and *Tectona grandis* (10%) in HPMC (7.5%) was used as high dose (TG 10%) for topical application in excision, incision and burn wound models. *Tectona grandis* 250 mg/kg (TG 250 mg/kg *p.o*) and 500 mg/kg weights (TG 500 mg/kg *p.o*) were administered orally in the dead space wound model. 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 scar falling.

Effect on excision wound (Kamath *et al*., 2003; Morton and Malone, 1972)

The animals were anesthetized by using pentobarbitone (30 mg/kg *i.p*). 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 treated as follows: Group I: 2% Tween 80 in HPMC gel (control), Group II: *Aloe vera* (90%) gel formulation, Group III: TG 5%, Group IV: TG 10%.

Wound area was measured by tracing the wound on a millimeter scale graph paper. The percentage of wound healing was calculated of original wound size (500 mm^2) for each animal of group on predetermined days i.e., 2, 4, 8, 10, 12, 14, 16, 18, 22, 22 days post-wounding for final analysis of results. Falling of scar 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.

Effect on incision wound (Lee, 1968; Ehrich and Hunk, 1969; Somayaji *et al*., 1995)

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 wound was closed by means of interrupted sutures placed at equidistance points about 1 cm apart. Animals were treated daily with drugs, as mentioned above under excision wound model from 0 day to 9^{th} post-wounding day. The wound breaking strength was estimated on 10^{th} day by continuous, constant water flow technique.

Effect on burn wound (Holla *et al*., 1968; Rao *et al*., 2000)

Partial thickness burn wounds were inflicted on overnight-starved animals under pentobarbitone (30 mg/kg, *i.p*.) anesthesia by pouring hot molten wax at 80 ^0C. 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 vehicle was applied topically as mentioned above.

Effect on dead space wound model (Padmaja *et al.*, 1994)

This type of wound was created by implanting subcutaneously a 2.5x0.5 cm polypropylene tube in the lumber region of dorsal side in anesthetized rats. Animals received one of the following treatments from 0 day to 9^{th} post wounding day. Group I: 2% Tween 80 solution (control), Group II: TG (250 mg/kg *p.o*), Group III: TG (500 mg/kg *p.o*). On the 10^{th} post wounding day, granulation tissue harvested on the implanted tube, was carefully dissected out along with the tube. The tubular granulation tissue was cut along its length 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°C for 24 hr to get a constant weight and weighed. The tissue was then used for the determination of hydroxyproline content (Neuman and Logan, 1950).

Skin irritation study (Gfeller *et al.,* 1985)
This study was carried out on rabbits. The skin of the animal was shaved at three different positions on the dorsal side, each about 500 mm^2. The 1st area was kept as control, to which vehicle was applied. 2nd area was applied with TG (5%) and the 3rd area treated with TG (10%). After 4 hr, the skin was observed for signs of inflammation.

STATISTICAL ANALYSIS

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

RESULTS

Preliminary phytochemical investigation
The preliminary phytochemical investigation of TG revealed the presence of carbohydrate, tannins and anthraquinone glycosides.

Selection of gel base
The result showed that both the rate and extent of tannin delivered from the vehicle under sink condition was maximum for the gel prepared with HPMC. There was no apparent retarding effect of the gel on the active moieties; hence HPMC was selected as vehicle for the formulation of extract (fig. 1).

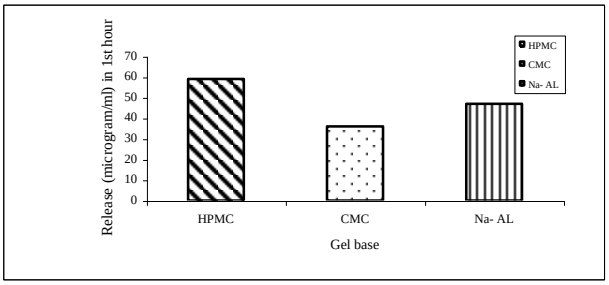

Fig. 1: Release of tannin from various gel base in first hour.

Acute oral toxicity study
The TG was safe at a dose of 2000 mg/kg body weight orally. Hence 1/4th and 1/8th of this dose i.e. 500 mg/kg and 250mg/kg body weight were used to study the effect in dead space wound model.

Effect on excision and incision wound
Both TG (5%) and TG (10%) produced a significant decrease in period of epithelization when compared to control ($P<0.001$). Treatment with *Aloe vera* also produced significant reduction in the period of epithelization ($P<0.001$). All treatment also showed significant decrease in wound contraction (50%) as compared to control. Competitive analysis revealed that TG (5%), TG (10%) and *Aloe vera* had almost equal wound healing activity. In the incision wound model, all the three treatments produced a significant increase ($P<0.001$) in the breaking strength of the wound (table 1).

Table 1: Effect of *Tectona grandis* on period of epithelization and wound contraction in excision wound model and breaking strength in incision wound model.

Group	Excision wound		Incision wound
	Epithelization period (days)	Wound contraction -50% (days)	Breaking strength
Control (7.5% HPMCgel)	21.00 ± 0.894	8.608 ± 1.120	283.33 + 20.412
Aloe vera gel (90%)	15.83 ± 1.329***	6.660 ± 0.919**	391.66 ± 40.825***
T.G (5%) in 7.5% HPMC gel	15.00 ± 1.414***	5.72 ± 0.650***	400.00 ± 15.811***
T.G.(10%) in 7.5% HPMC gel	16.00 ± 1.265***	6.07 ± 0.347***	370.83 ± 24.580***

All values are mean ± SD, n=6, **P<0.01, ***P<0.001 vs. control.

Table 2: Effect of *Tectona grandis* on period of epithelization and wound contraction in burn wound model.

Parameter studied	Epithelization period (days)	Wound contraction -50% (days)
Control (7.5% HPMC gel)	20.50 ± 1.225	7.87 ± 0.784
Aloe vera gel (90%)	16.16 ± 0.983***	5.37 ± 0.675***
T.G. (5%) in 7.5% HPMC gel	14.33 ± 1.566***	4.31 ± 0.551***
T.G. (10%) in 7.5% HPMC gel	16.50 ± 1.643***	5.22 ± 0.67²***

Table 3: Effect of *Tectona grandis* on breaking strength, dry tissue weight and hydroxyproline content in dead space wound model.

Group	Breaking strength	Dry tissue weight	Concentration of hydroxyproline
Control	337.5 ± 41.563	90.56 ± 6.359	2933.33 ± 326.60
T.G 250mg/kg	597.5 ± 44.244***	189.16 ± 14.746***	6066.66 ± 467.62***
T.G 500mg/kg	636.6±34.881***	185.58 ± 14.076***	7600.00 ± 669.33***+++

All values are mean ± SD, n=6, *** $p<0.001$ vs. control +++ $p<0.001$ vs. low dose.

Effect on burn wound

Application of TG (5%), TG (10%) and *Aloe vera* gel topically shortened the period of epithelization significantly ($P<0.001$) and also produced a significant decrease ($P<0.001$) in wound contraction (50%) when compared to control (table 2).

Effect on dead space wound

The breaking strength of 10 days old granulation tissue was significantly promoted ($P<0.001$) by TG (250 mg/kg, *p.o*) and TG (500 mg/kg, *p.o*). The dry tissue weight was significantly increased by both TG (250mg/kg, *p.o*) and TG (500mg/kg, *p.o*) treatment when compared with control ($P<0.001$). The hydroxyproline content was significantly more ($P<0.001$) in TG high dose treated animal compared to T.G. low dose treated animal (table 3).

Skin irritation study

TG (5%) did not show any severe type of irritation and there was no evidence of any noticeable inflammation but slight redness was observed. On the other hand, TG (10%) showed well-defined redness and moderate inflammation (table 4).

Table 4: Skin irritation study

Group	Sign	Score
Control	--	0
Tectona grandis (5%)	No noticeable inflammation but slight redness	0.5
Tectona grandis (10%)	Well defined redness and moderate type of inflammation	3.5

DISCUSSION

The present study was undertaken to evaluate whether *Tectona grandis* leaf extract could promote wound healing in experimentally produced wounds in rats. The results of the present study substantiate the use of *Tectona grandis* leaves in folklore medicine for the treatment of wounds. The extract applied topically or given orally 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 phase inflammation, macrophasia, fibroplasias and collagenation are intimately interlinked. Thus an intervention into any one of these phases by drugs could eventually lead to either promotion or depression of the collagenation phase 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. Collage is synthesized by a complex biochemical mechanism of ribosome. The collagen synthesis is stimulated by various growth factors (Corton *et al.,* 2003). Growth hormone is also known to promote the proliferation of fibroblasts (Williams and Frohman, 1986) and fibroblast proliferation form the granulation tissue. In the dead space wound model *Tectona grandis* treatment increased granuloma tissue weight and breaking strength. Hence it is assumed the pro-healing activity of *Tectona grandis* could be due to the direct or indirect influence on growth hormone release.

Tectona grandis contains tannin, which are used as anti-inflammatory agents and also used topically for treatment of burns (Evans, 1998; Shah *et al.,* 1995). 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. Antioxidants such as metronidazole, vitamin C, vitamin E have been shown to promote wound contraction and epithelization (Rao and Ghosh, 1997). The antioxidant property of the *Tectona grandis* leaves, conferred upon it by the presence of high amounts of tannin may also be responsible to the prohealing action of the extract.

The skin irritation study on the rabbit skin proved that TG (10%) produce irritation to the skin while TG (5%) did not show any severe type of irritation. This suggests that

the drug may contain some chemical constituents, which does not produce significant irritation at low dose but at high concentrations may cause irritation and navigate the wound healing activity of *Tectona grandis*. This explains why *Tectona grandis* low dose showed slightly higher activity than the high dose when applied topically but not when administered orally. Further studies have to be carried out to determine the skin toxicity of the plant and to identify the active constituent(s) responsible for skin irritation before claiming the drug to be a safe wound healing agent.

Since *Tectona grandis* is more potent than *Aloe vera* in excision, incision, burn and dead space wound, isolation of the active principle from the extract and removal of the constituent(s) responsible for skin irritation, may lead to the development of a wound healing agent that may turn out to be a promising agent in not only open wound but also leg ulcer, skin grafting and severe type of burn.

To conclude, the hydro-alcoholic extract of *Tectona grandis* exhibited significant wound healing activity in excision, incision, burn and dead space wound model. The effect observed in first three was comparable to that of marketed *Aloe vera* gel formulation. However, the extract in higher doses produced skin irritation.

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