

Acceleration of Wound Healing Potential by *Lantana camara* Leaf Extract in Experimental Rats

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Abstract: The ethanolic extract of *Lantana camara* leaf was evaluated for their wound healing potential in rats. Four groups of adult male Wistar albino rats were experimentally wounded in the posterior neck area. A thin layer of blank placebo was applied topically to wounds of Group 1. Wound of Group 2 and 3 animals were dressed with a thin layer of placebo containing 5 and 10% *L. camara* extract, respectively. A thin layer of intrasite gel was applied topically to wounds of Group 4 animals as reference. The effects of these topical applicants on the rate of wound healing and histology were assessed. Wound dressed with placebo containing plant extracts significantly healed earlier than those treated with blank placebo. Wounds dressed with placebo containing 10% extract significantly accelerate wound healing activity compared to wounds dressed with placebo containing 5% extract. Histological analyses of healed wounds confirmed the results. Wounds dressed with placebo containing extracts showed markedly less scar width at the wound enclosure and wounds contained large amounts of fibroblast proliferation and more mature and densely packed collagen with accompanying angiogenesis compared to wounds dressed only with blank placebo. We conclude that *L. camara* extract significantly enhanced the acceleration rate of wound enclosure in rats.

Key words: *Lantana camara*, aqueous extract, wound healing, histology, placebo, intrasite gel

INTRODUCTION

Lantana camara L. (Verbenaceae) is listed as one of the important medicinal plants of the world (Ross, 1999). *Lantana* plant has been reported to possess a number of medicinal properties (Ghisalberti, 2000). Various parts of the plant are used in the treatment of itches, cuts, ulcers, swellings, bilious fever, catarrh, eczema, dysentery and chest complaints of children, fistula, pustules and rheumatism (Agarwal, 1997). Some metabolites isolated from their leaves have antitumor activity (Mahato *et al.*, 1994); inhibitor of protein kinase C (Herbert *et al.*, 1991) antiviral (Inada *et al.*, 1995); antinociceptive and antipyretic activity (Uzcategui *et al.*, 2004); antithrombin activity (O' Neill *et al.*, 1998); antimicrobial (Kumar *et al.*, 2006); antimutagenic (Barre *et al.*, 1997); antimotility (Sagar *et al.*, 2005); antiinflammatory (Basu and Hazra, 2006); insecticidal and termicidal effects (Bouda *et al.*, 2001; Verma and Verma, 2006) and antioxidant activity

(Saini *et al.*, 2007). There are no data available regarding its wound healing potential of *L. camara* leaves. Therefore, the present study was undertaken to evaluate the wound healing property of ethanolic leaf extract in rats.

MATERIALS AND METHODS

Placebo: An aqueous cream placebo was obtained from Department of Pharmacy, Faculty of Medicine, University of Malaya (Sunward Pharmaceutical SDN BHD. MAL 19920890X).

Intrasite gel: Intrasite gel was purchased from University Malaya Medicinal center Pharmacy. Intrasite gel is a colorless transparent aqueous gel, which contains a Modified Carboxymethylcellulose (CMC) polymer together with propylene glycol as a humectant and preservative. Intrasite gel, is an amorphous hydrogel,

which gently re-hydrates necrotic tissue, facilitate autolytic debridement, while being able to loosen and absorb slough and exudates, clearing the way for effective wound healing. It is also designed for wounds that are granulating and epithelialising. It can also be used to provide the optimum moist wound management environment during the later stages of wound closure. It is non-adherent and does not harm viable tissue or the skin surrounding the wound. This makes Intrasite gel ideal for every stage in the wound management process. (Intrasite gel is a trademark for Smith and Nephew Ltd.) (Williams, 1994).

Lignocaine HCl (2%, 100 mg/5 mL): The local anesthesia was purchased for Animal House, Faculty of Medicine, University Malaya.

Plant materials: The leaf of *Lantana camara* were collected from University Malaya garden and identified by comparison with specimens available at the Herbarium of Rimba Ilmu, Institute of Science Biology University Malaya. The plants were, wash with water and dried in incubator at 50°C for 5-7 days. The dried materials were ground to powder using a grinder.

Preparation of *L. camara* alcoholic extract: The dried powder was extracted by maceration in ethanol (100 g/1500 mL, w/v⁻¹) in a conical flask for 5 days at 37°C. Afterwards, the solvent was filtered using a filter funnel and the solvents were distilled under reduced pressure in EYELA rotary evaporator until excess solvent evaporated. The alcoholic extract of *L. camara* was mixed homogeneously with blank Vaseline in the concentration of 5% and 10% (w w⁻¹) each. The extracts were kept in fridge at 4°C until used.

Experimental animals: Wistar albino rats were obtained from the animal house, Faculty of Medicine, University of Malaya and Ethic No. PM 28/9/2006 MAA (R). The rats were divided randomly into 4 groups of 6 rats each. Each rat that weighted between 200-250 g was housed separately. The animals were maintained on standard pellet diet and tap water.

Experimentally induced wounds: The animals were anesthetized by diethyl ether. The skin shaved, disinfected with 70% alcohol and injected with 1 mL of Lignocaine HCl (2%, 100 mg/5 mL). An circle area of uniform wound 2.00 cm in diameter was excised from the nape of the dorsal neck of all rats with the aid of round seal as described by Morton and Melone (1972) with slight modification (Fig. 1). Avoid incision of the muscle layer and tension of skin was kept constant during the procedure.

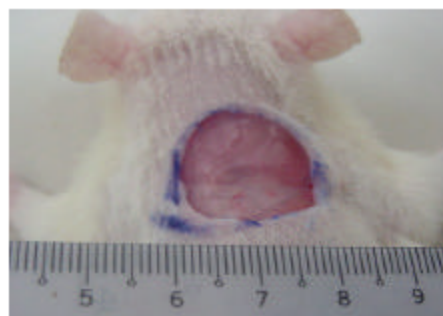


Fig. 1: 2.00 cm diameter excision skin wound on day 0 before application of vehicle

Topical application of vehicles: Group 1 animals were dressed topically twice daily with thin layer of blank placebo. Thin layer of placebo containing 5 and 10% *Lantana camara* extracts each were applied topically twice daily to the wounds of Group 2 and 3 rats, respectively. Thin layer of Intrasite gel were topically applied twice daily to Group 4 animals as reference. The wound was observed daily until complete wound-healing process occurs and then the animals were sacrificed by overdose of diethyl ether.

Histological evaluation of healed wounds: Specimens of skin from healed wounds from each rat were fixed in 10% buffered formalin solution for histopathological studies. Sections of the healed skin were made at a thickness of 5 μ L, stained with hematoxylin and eosin (H and E), were assessed for histopathological changes. The microscopic slides were photographed.

Statistical analysis: All values are reported as mean \pm SEM and the statistical significance of differences among groups were assessed using one-way ANOVA. A value of $p < 0.05$ was considered significant.

RESULTS

Wounds treated with alcoholic extracts of *L. camara* or with Intrasite gel showed considerable signs of dermal healing and significantly ($p < 0.05$) decrease mean wound healing time than those treated with blank placebo (Table 1, Fig. 2 and 3). Also wounds treated with 10% extract or Intrasite gel significantly possesses better healing and healed faster compared to wounds treated with 5% extract in placebo. There were no significant differences between wounds treated with 10% *L. camara* extract and Intrasite gel in the term of duration of wound healing (Table 1).

Table 1: Time required for wound healing by *L. camara* in experimental animals

Animal groups	No. animals	Type of dressings	Healing time (days)
1	6	Blank placebo (Negative control)	19.83±0.67 ^a
2	6	5% <i>L. camara</i> in placebo	15.17±0.48 ^b
3	6	10% <i>L. camara</i> in placebo	12.17±0.48 ^c
4	6	Intrasite gel (positive control)	12.00±0.37 ^c

All values were expressed as mean and ±Standard Error Mean; Mean with different superscripts were significantly different (p<0.05)

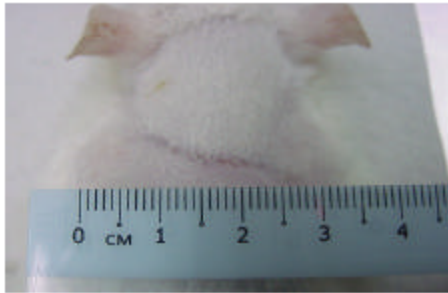


Fig. 2: Complete wound healing with 10% *L. camara* extract on day 11

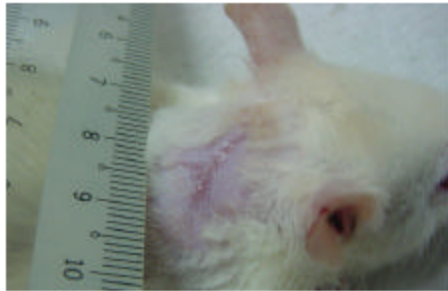


Fig. 3: Wound healing on day 19 in animal treated with blank placebo

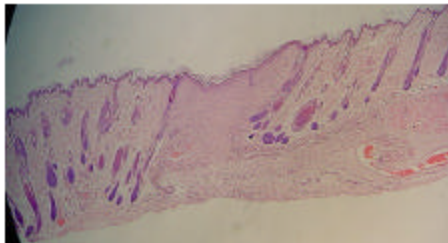


Fig. 4: Histological section of a healed wound treated with 10% *L. camara* extract showing an intermediate scar region (H and E stain ×40)

Histologically, wounds dressed with methanolic extracts of *L. camara* or Intrasite gel contained markedly fewer inflammatory cells, less scarring at the wound enclosure, more proliferating blood capillaries (angiogenesis) and more collagen fibres compared to wounds dressed only with blank placebo (Fig. 4-7).

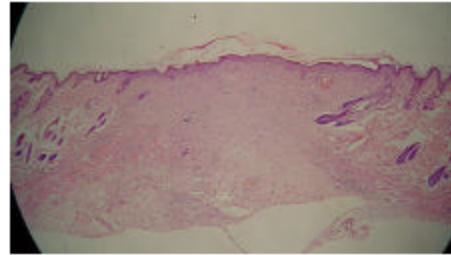


Fig. 5: Histological section of a negative control wound showing a very broad scar region (H and E stain ×40)

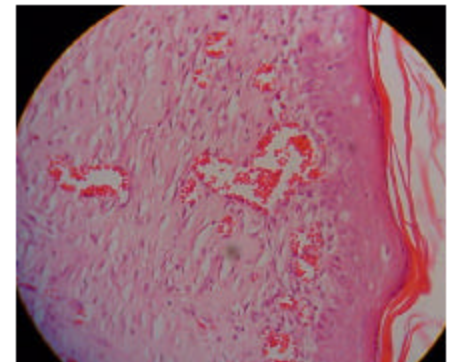


Fig. 6: Histological section of a healed wound dressed with placebo containing 10% *L. camara* extract. Granulation tissue contains more collagen, fibroblast and blood capillaries and there is an absence of inflammatory cells (H and E stain ×80)

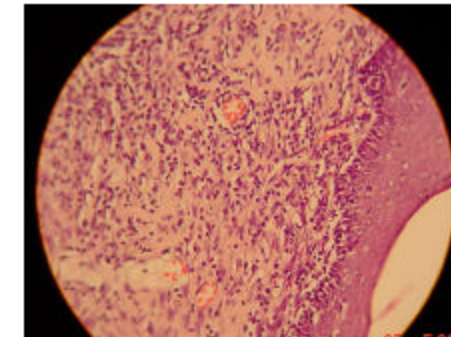


Fig. 7: Histological section of a healed wound dressed only with blank placebo. Granulation tissue contains less collagen, fibroblast and blood capillaries and more inflammatory cells (H and E stain ×80)

DISCUSSION

In the present study, topical application of *L. camara* extracts significantly enhanced the rate of wound healing. Wound healing effects may be due to up-regulation of human collagen I expression (Bonte *et al.*, 1993) and an increase in tensile strength of the wounds (Suguna *et al.*, 1996). Enhanced healing activity has been attributed to increased collagen formation and angiogenesis (Shukla *et al.*, 1999). Collagen occupies a central role in the healing of wounds, as it is a principal component of connective tissue and provides a structural framework, strength and milieu for the regenerating tissue (Cohen *et al.*, 1992). Angiogenesis in granulation tissues improves circulation to the wound site thus, providing oxygen and nutrients essential for the healing process (Szabo *et al.*, 1995) that include re-epithelization. Growth factors such as basic fibroblast growth factor stimulate epithelial cell proliferation and angiogenesis thus are important to accelerate wound healing activity (Buntrock *et al.*, 1982). Oxidative stress plays a major role in the pathogenesis of various diseases including wounds and ulcers. *L. camara* leaf extract has shown to contain flavonoids (Pan *et al.*, 1993) and terpenes (Begum *et al.*, 2003), which may mediate the wound healing process. *L. camara* prevented lipid peroxidation that may suggest that it may be acting as antioxidant (Saini *et al.*, 2007). Antioxidants have been reported to play a significant role in the wound healing process. It appears that antioxidant may be an important contributory factor in the wound healing property (Shukla *et al.*, 1999). Studies have shown the topical application of compounds with antioxidant properties on patients or animals to significantly improve wound healing and protect tissues from oxidative damage (Martin, 1996). *L. camara* leaf possesses antitumor, antiviral, antibacterial and anti-inflammatory activities and thus the wound healing activity could be attributed to these properties (Xu *et al.*, 1996). Wound healing properties of *L. camara* also could be due to antimicrobial activity (Deena and Thoppil, 2000). *L. camara* extract exhibited significant antimicrobial activity and properties that support folkloric use in the treatment of some diseases as broad spectrum antimicrobial agents. This probably explains the use of these plants by the indigenous people against a number of infections since generation (Kumar *et al.*, 2006).

CONCLUSION

In conclusion, the current study revealed that methanolic extracts of *L. camara* were suitable as topical application for wounds as indicated by the significantly

faster rate of healing and reduced scarring at the wound enclosure. Histologically, there were also comparably fewer inflammatory cells, more angiogenesis and collagen fibres in the granulation tissue.

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