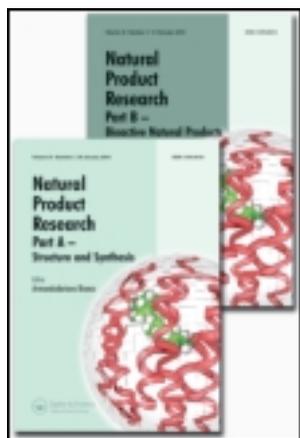


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## Effect of *Andrographis paniculata* leaf extract on wound healing in rats

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This work was carried out to study the effect of topical application of *Andrographis paniculata* on the rate of wound enclosure and its histological features. A wound was created in four groups of rat in posterior neck region. Blank placebo was applied topically to the wounds of Group 1. Groups 2 and 3 were dressed with placebo containing 5% and 10% extracts of *A. paniculata*, respectively. Intrasite gel was applied topically to the wounds of Group 4. Macroscopical examination revealed that the rate of wound healing was significantly accelerated in the wound dressed with *A. paniculata* extract compared to the blank placebo. The wounds dressed with 10% extract or Intrasite gel healed earlier compared to the wounds dressed with placebo containing 5% *A. paniculata* extract. Histologically, wounds dressed with *A. paniculata* extracts showed markedly less scar width and contained large amounts of fibroblast proliferation. More collagen and less angiogenesis with absence of inflammatory cells were seen for wounds dressed with 10% *A. paniculata* compared to the blank placebo. Conclusion, *A. paniculata* extracts significantly enhanced rate of wound healing in rats.

**Keywords:** *Andrographis paniculata*; wound healing; histology

### 1. Introduction

*Andrographis paniculata* Nees belongs to the family Acanthaceae, which is widely used in traditional medicine in Southeast Asia and China to treat various diseases, including diabetes, hypertension and cancer (Ajaya, Sridevi, Kumar, Nanduri, & Rajagopal, 2004). Their leaves are known to contain diterpenes, flavonoids, stigmaterols, andrographolide together with several other diterpenoids (Siripong et al., 1992). Herbal extracts of *A. paniculata* are useful as anti-inflammatory (Sheeja, Shihab, & Kuttan, 2006), antioxidant (Tripathi & Kamat, 2007), antiviral (Calabrese et al., 2000), anticancer (Ajaya et al., 2004), antimicrobial (Singha, Roy, & Dey, 2003), antimalarial (Siti Najila et al., 2002),

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and are hepatoprotective (Trivedi, Rawal, & Patel, 2007) agents. It had also shown immunostimulatory (See, Mason & Roshan, 2002), phagocytotic (Matsuda et al., 1994), anti-diabetic (Reyes et al., 2006) and hypotensive (Zhang & Tan, 1996) activities. However, there is no data available regarding wound-healing capability from *A. paniculata* leaf extracts. The present study was undertaken in rats to evaluate the wound-healing potential from aqueous extracts of *A. paniculata* leaves.

## 2. Results

### 2.1. Wound-healing activity

The wounds dressed with either aqueous extracts of *A. paniculata* leaves or with Intrasite gel showed considerable signs of dermal healing, and significantly ( $p < 0.05$ ) healed earlier compared to the wounds dressed with blank placebo (Table 1, Figure S1). The wounds dressed with Intrasite gel or 10% *A. paniculata* extracts significantly possessed better healing and healed faster compared to wounds treated with 5% *A. paniculata*. There were no significant differences between wounds dressed with 10% *A. paniculata* extract and Intrasite gel in terms of duration of wound healing enclosure (Table 2). Histologically, wounds dressed with aqueous

Table 1. Time required for wound healing by *A. paniculata* in experimental animals.

Animal groups	No. of animals	Type of dressings	Healing time (days) (mean $\pm$ SEM)
Group 1	6	Blank placebo	19.17 $\pm$ 0.63 <sup>a</sup>
Group 1	6	Placebo containing 5% <i>A. paniculata</i>	14.83 $\pm$ 0.48 <sup>b</sup>
Group 3	6	Placebo containing 10% <i>A. paniculata</i>	12.33 $\pm$ 0.21 <sup>c</sup>
Group 4	6	Intrasite gel (positive control)	12.67 $\pm$ 0.21 <sup>c</sup>

Note: All values were expressed as means  $\pm$  SEM. Mean with different superscripts were significantly different ( $p < 0.05$ ).

Table 2. Effect of *A. paniculata* on percentage wound healing in experimental rats.

Animal groups	Vehicles	Percentage wound healing (means $\pm$ SEM) on day post surgery				
		1	5	10	15	20
Group 1	Blank placebo (negative control)	0.0	38.17 $\pm$ 0.6 <sup>a</sup>	65.17 $\pm$ 0.95 <sup>a</sup>	83.17 $\pm$ 0.83 <sup>a</sup>	100 $\pm$ 0.00
Group 2	Intrasite gel (positive control)	0.0	75.17 $\pm$ 1.51 <sup>b</sup>	97.17 $\pm$ 0.95 <sup>b</sup>	100 $\pm$ 0.00 <sup>b</sup>	100 $\pm$ 0.00
Group 3	Placebo containing 5% <i>A. paniculata</i>	0.0	68.67 $\pm$ 1.09 <sup>c</sup>	89.00 $\pm$ 0.82 <sup>c</sup>	98.00 $\pm$ 0.58 <sup>b</sup>	100 $\pm$ 0.00
Group 4	Placebo containing 10% <i>A. paniculata</i>	0.0	72.17 $\pm$ 0.95 <sup>bc</sup>	92.17 $\pm$ 0.95 <sup>c</sup>	100.00 $\pm$ 0.00 <sup>b</sup>	100 $\pm$ 0.00

extracts of *A. paniculata* leaves 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 with sterile deionised water or blank placebo (Supplementary Figures S2 and S3 – online only).

### 3. Discussion

*Andrographis paniculata* has been reported to possess antioxidant activities (Trivedi & Rawal, 2001). Antioxidants have been reported to play a significant role in improving the wound-healing process and protecting the tissues from oxidative damage (Martin, 1996). Phytochemical analysis of the *A. paniculata* extract showed the presence of several bioactive molecules such as flavonoids and andrographolides (Koteswara, Vimalamma, Venkata Rao, & Tzeng, 2004). These phytochemical constituents may be responsible for the wound-healing activity. Flavonoids are known to promote the wound-healing process due to the antimicrobial properties, which appear to be responsible for the wound contraction and increased rate of epithelialisation (Tsuchiya et al., 1996). Additionally, *A. paniculata* leaf extract contains antimicrobial activity (Singha et al., 2003) to which the wound-healing properties of *A. paniculata* could be attributed. Wound-healing mechanisms may be contributed to stimulate the production of antioxidants in wound site and to provide a favourable environment for tissue healing (Shukla, Rasik, & Dhawan, 1999); wound-healing effects may be due to the up-regulation of human collagen I expression (Bonte, Dumas, Chadgne, & Meybeck, 1993) and due to an increase in tensile strength of the wounds (Suguna, Sivakumar, & Chandrakasan, 1996). Enhanced healing activity has been attributed to increased collagen formation and angiogenesis (Shukla et al., 1999; Trabucchi, Preis-Baruffaldi, Baratti, & Montorsi, 1986). 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-epithelisation. Stimulation of epithelial cell proliferation and angiogenesis are important for wound-healing process (Buntrock, Jentzsch, & Heder, 1982). Habibipour et al. (2003) showed that treated healed wound group contained a large amount of fibroblast proliferation, collagen synthesis and neovascularisation, which resulted in an increased wound tensile strength and accelerated healing wound. In conclusion, the current study revealed that dressing with *A. paniculata* extracts as topical application of wounds significantly enhanced the wound-healing process.

### 4. Experimental

#### 4.1. Placebo

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

#### **4.2. *Intrasite gel***

Intrasite gel was purchased from the University Malaya Medicinal Centre Pharmacy. Intrasite gel is a colourless transparent aqueous gel which contains a modified carboxymethylcellulose (CMC) polymer together with propylene glycol as a mucoctant and preservative. Intrasite gel is an amorphous hydrogel which gently re-hydrates necrotic tissue and facilitates autolytic debridement while loosening and absorbing 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 the use of Intrasite gel ideal for every stage in the wound management process. (Intrasite gel is a trademark for Smith and Nephew Ltd) (Williams, 1994).

Lignocaine is a local anaesthesia and was purchased from the Experimental Animal House, Faculty of Medicine, University of Malaya (Delta Veterinary Laboratory PTY LTD, NSW 20011). One millilitre of Lignocaine was injected subcutaneous.

#### **4.3. *Plant specimen and extract preparation***

Fresh *A. paniculata* leaves were collected in Johor, Malaysia, and identified by comparison with the voucher specimen deposited at the Herbarium of Rimba Ilmu, Institute of Science Biology, University of Malaya, Kuala Lumpur. The leaves were washed with distilled water, dried in shade for 7–10 days and ground to powder. Four hundred grams of the leaf powder was added to 8000 mL of sterile distilled water (1 : 20) in a conical flask. This mixture was heated and stirred on a hotplate for 3 h. The residue was removed by filtration using a filter funnel and distilled under reduced pressure in an Eyela rotary evaporator (Sigma-Aldrich, USA). The extract was then freeze-dried to produce powdered forms of the extract. The freeze-dried products were mixed homogeneously with blank placebo in concentrations of 5% and 10% (w/w).

#### **4.4. *Experimental animals***

Adult male albinos Wistar rats were obtained from the Animal House, Faculty of Medicine, University of Malaya, Kuala Lumpur (Ethics No. PM 28/9/2007 MAA (R)). The rats were divided randomly into four groups of six rats each. Each rat that weighted between 200 and 230 g was housed separately (one rat per cage). The animals were maintained on standard pellet diet and tap water. The procedures involving animals and their care conformed to the international guideline, Principles of Laboratory Animals Care.

#### **4.5. *Experimentally induced wounds***

Initially, the animals were anaesthetised with diethyl ether. The skin of the posterior neck region was then shaved with an electrical shaver, disinfected with 70% alcohol and injected with 1 mL of Lignocaine HCl (2%, 100 mg per 5 mL). A uniform wound

area of 2.00 cm in diameter was excised from the nape of the dorsal neck of all rats aseptically with the aid of round seal as described by Morton and Melone (1972) with slight modifications (Supplementary Figure S1 – online only). Incision of the muscle layer was avoided by keeping the tension of skin constant during the procedure.

#### **4.6. Topical application of vehicles**

Wounds of Group 1 rats were dressed with a thin layer of blank placebo twice daily, while the wounds of Group 2 and 3 animals were treated topically with a thin layer of placebo containing 5% and 10% plant extract twice daily, respectively. A thin layer of commercial Intrasite gel was topically applied twice daily to the wounds of Group 4 rat as reference.

#### **4.7. 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  $\mu$ L, stained with haematoxylin and eosin (H & E), were assessed for histopathological changes. The microscopic slides were photographed.

#### **4.8. Histological analysis for quantitating angiogenesis and inflammatory cells under light microscopy**

Three tissue sections from the healed wound area from each animal was analysed for counting the inflammatory cells and to measure the blood vessels' area using light microscopy under 40 $\times$  with a planimeter grid in 0.15 square area covering the centre of healed wound and adjacent areas on both sides (three big squares for each tissue section/animal).

#### **4.9. Rate of wound-healing closure**

Blinding was done randomly to assess the rate of wound-healing closure by other author who did not know the grouping (labelling) of animal's treatment.

#### **4.10. Estimation of wound healing (wound closure)**

Wound areas were traced manually and calculated in square millimetres. The wound closure area of each animal was assessed by tracing the wound on days 1, 5, 10, 15 and 20 post-wounding surgery, and the wound closure rate was expressed as the percentage of wound area compared with that on post-operative day by using transparency paper and a permanent marker under light diethyl ether anaesthesia as described by Nayak and Pinto-Pereira (2006) with slight modification. The wound areas recorded were measured using a graph paper. The percentages of wounds healing on these days were determined (Chah, Eze, Emuelosi, & Esiomone, 2006).

The number of days required for falling of scar without any residual raw wound gave the period of epithelisation.

#### 4.11. Statistical analysis

All values were reported as mean  $\pm$  standard errors mean (SEM). The statistical significance of differences between groups was assessed using one-way ANOVA. A value of  $p < 0.05$  was considered significant.

#### Supplementary material

Figures S1–S3 relating to this paper are available online.

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