Indian J Physiol Pharmacol 2005; 49 (4): 487–490

LETTER TO THE EDITOR

EFFECT OF *PHYLLANTHUS NIRURI* ON WOUND HEALING IN RATS

Sir,

(Received on June 1, 2005)

Phyllanthus niruri (P. niruri) Schum and Thonn syn. *P. amarus* Linn (Bhumyamalaki in Sanskrit) is a herb, occurring as a winter weed usually, throughout the hotter part of India, particularly in cultivated land (1).

P. niruri is commonly known for its usefulness in the treatment of jaundice (1). Extensive studies on *P. niruri* have confirmed this plant preparation as being anti-viral against hepatitis B and C viruses, hepatoprotective, immunomodulating as well as possessing anti-inflammatory properties (2). This plant has been evaluated for diuretic, hypotensive, hypoglycemic (1), antioxidant (3), antibacterial (4) and analgesic (5) properties. The analgesic property has been attributed to a phytochemical geranin (6) which has also been reported to have antiulcerous and gastroprotective properties (7). This herb also has got inhibitory effect on renal stone growth (8).

P. niruri is reported to be useful in the treatment of skin ulcers, wounds, sores, swelling and itchiness (9). A poultice of the leaves and roots in rice water is used in edematous swelling and ulcers (10). Since the plant has been reported to posses antioxidant (3) and antiulcerous (7) properties and survey of literature revealed that no systematic approach has been made to study wound healing activity of this plant, it was thought worthwhile to investigate if alcoholic extract of *P. niruri* could promote healing in experimentally induced wounds in rats. If so, whether it could reverse the dexamethasone depressed wound healing.

The whole plants of *P. niruri* were procured from a local shop during September 2002 and authenticated by the department of Botany, M.G.M College, Udupi.

The whole plant of *P. niruri* was dried under shade and then macerated. 500 g of *P. niruri* was soaked in 3 litres of absolute alcohol (Department of pharmacology, Manipal). After 24 hours, extraction was started by reflux condensation. The extract was concentrated by distillation and then evaporated to dryness on a waterbath. 500 g of dried *P. niruri* yielded 85.45 g of the alcoholic extract. The dose of *P. niruri* was fixed at 200 mg/kg body weight of the rat after doing acute toxicity studies and this was the dose used by earlier workers (3). The suspension of alcoholic extract of *P. niruri* was made in 2% gum acacia (E. Merck limited, Mumbai) in such a way that 1 ml of the suspension contained 40 mg of the extract.

Twelve week old healthy, male albino rats of Wistar strain, weighing between 150–

200 g were used. The study protocol was approved by the institutional animal ethical committee. Rats were individually housed under controlled conditions of temperature of 23±2°C, humidity of 50±5% and maintained on normal diet (Hindustan Lever rat pellets) and water *ad libitum*.

Three wound healing models were selected for assessing the wound healing activity. Four groups of animals containing eight animals each per model were used. The control group received 2% gum acacia daily orally. The remaining groups received respectively *P. niruri* (orally), dexamethasone (Cadila Healthcare, Mumbai) (0.17 mg/kg im) (11) and the combination of *P. niruri* and dexamethasone. *P. niruri* and dexamethasone were administered once daily from day 0 to day 9 in incision and deadspace wound models and from day 0 to the day of complete healing or the 21st post operative day, whichever was earlier in excision wound model.

The animals were starved overnight prior to wounding. The surgical procedures were done under ether anaesthesia. After the study period the animals were sacrificed by giving pentobarbitone (50 mg/kg, ip) prior to determination of the tensile strength of incision wounds and for the removal of granulation tissue from dead space wounds.

Incision wound was created by putting two paravertebral incisions of 6 cm through the entire thickness of the skin, on either side of the vertebral column on each rat (12), and edges were approximated by interrupted sutures at 1 cm intervals. The sutures were removed on day 7. On day 10, the breaking strength of the wound was measured by continuous waterflow technique of Lee (13).

The dead space wound was created by implanting subcutaneously, 2.5 × 0.5 cm polypropylene tube in the lumbar region, on the dorsal side, through a small transverse incision on each rat (12). On the 10th post wound day, the granulation tissue harvested on the implanted tube was dissected out along with the tube. The breaking strength of granulation tissue was measured. Then the pieces of granulation tissue were dried to 60°C for 24 h to get a constant weight and then weighed. After noting down the dry weight, granulation tissue were used for the determination of the hydroxyproline content (expressed as mg/g of the tissue) by the method of Neuman and Logan, 1950 (14).

An excision wound was made by cutting away the full thickness skin of approximately 500 mm^2 from the predetermined area on the dorsal interscapular region of each rat (12). Wound contraction rate was monitored by tracing the raw wound area on a transparent paper on 4th, 8th, 12th and 16th post wound days. Reduction in wound area was expressed as % of original wound area (500 mm^2). Period of epithelialisation was monitored by noting the number of days required for the scab to fall away leaving no raw wound behind.

Results were analysed by one way analysis of variance (ANOVA) followed by scheffe's test using SPSS computer package, P value <0.05 was considered significant.

Healing is a complex process which involves number of phases viz, coagulation, inflammation, collagenation, wound contraction and epithelialisation. While the phases between coagulation to collagenation are intimately interlinked, the phases of wound contraction and epithelialisation are independent of each other and run concurrently. No single model can thus be sufficient to assess the influence of drugs on wound healing.

Therefore, in the present study three wound healing models viz, incision, dead space (for collagenation phase) and excision (for wound contraction and epithelialisation phase) were employed.

The present study was conducted to evaluate the effect of *P. niruri* on wound healing. *P. niruri* per se did not significantly alter the wound healing parameters in incision and dead space wounds (Table I). In excision wound model, *P. niruri* promoted 4th day wound contraction but not on 8th, 12th, and 16th day as compared to control (Table–I).

To ascertain whether this wound healing promoting effect has any influence on steroid depressed wound, further study was conducted. *P. niruri* did not alter incision and dead space wound parameters (Table–I). But the noteworthy point is that, *P. niruri* significantly reversed dexamethasone suppressed wound contraction.

Clinically one may come across poor healing or non-healing. Poor healing may be due to infection or NSAIDs (15, 16) and steroids (17, 18). The latter are used to relieve

TABLE I : Wound parameters in incision, dead space and excision wounds (values are Mean±S.E.).

Drugs (n=8)	*Dose/Route*	*Incision*	*Dead space*			*Excision*				
						Wound contraction rate %				*Period of epithelialisation (days)*
		Breaking strength (g)	*Breaking strength of granulation tissue (g)*	*Dry wt. of granulation tissue (mg)*	*Hydroxyproline content of granulation tissue (mg/g)*	*4th day*	*8th day*	*12th day*	*16th day*	
Gum	2 ml oral	356.25± 28.66	273.33± 51.54	65± 18.05	0.68± 0.16	16.91± 1.67	80.27± 2.47	93.93± 0.9	96.57± 1.24	19.33± 1.22
P. niruri	200 mg/kg oral	376± 30.35	344.28± 42.64	91.42± 16.61	1.02± 0.18	39.7± 2.01[a]	84± 1.53	95.67± 1.03	96.13± 1.28	20.66± 0.82
Dexa	0.17 mg/kg im	217.83± 17.34[a]	163.33± 28.74	56.33± 11.07	0.34± 0.16	1.8± 0.93	16.95± 4.36[a]	51.1± 3.7[a]	78.93± 3.44[a]	21.66± 0.8
Dexa + *P. niruri*	0.17 mg/kg+ 200 mg/kg	311.83± 0.09	215.8± 29.64	58± 8.41	0.59± 0.10	20.75± 3.13[b]	44.93± 1.39[b]	77.6± 1.73[b]	86.1± 2.5	19± 0.45

a : P<0.05 Vs Control; b : P<0.05 Vs Dexamethasone; Dexa = Dexamethasone.

pain and inflammation associated with trauma or surgery. Since *P. niruri* has significantly reversed the steroid suppressed wound contraction, there could be a scope for *P. niruri* as a useful agent in the management of surgical or traumatic wounds.

VASUDHA DEVI*, TARA V. SHANBHAG, K. L. BAIRY, NAMITHA RAO AND SMITA SHENOY

Department of Pharmacology,
Kasturba Medical College,
Manipal – 576 104

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*Corresponding Author : E-mail : vasudha_manipal@yahoo.co.in