Natural Product Sciences
12(1) : 50-54 (2006)

Wound Healing Activity of the Chloroform Extract of *Plumbago rosea* Linn. and Plumbagin

A. Saraswathy[1,*], R.V. Pradeep Chandran[1], B. Murali Manohar[2], and S.Vairamuthu[2]

[1]Captain Srinivasa Murti Drug Research Institute for Ayurveda (CCRAS), Arumbakkam, Chennai-600 106, India
[2]Department of Pathology, Madras Veterinary College, Vepery, Chennai-600 007

Abstract – The wound healing activity of plumbagin and the chloroform extract of *Plumbago rosea* Linn. (Yoot), incorporated into ointments with yellow soft paraffin, have been investigated on rats. Wound healing activity was studied using excision and incision wound models in rats following topical application. Both plumbagin and the *Plumbago rosea* root extract produced a significant response in both of the wound models studied. The wound contracted in 14 days in the case of plumbagin (0.1%) and 16 days in case of *Plumbago rosea* root extract (0.5%), as against in 22 days in the case of control animals. The results were also comparable to those of a standard drug, framycetin sulphate cream (1% w/w) in terms of wound contracting ability, wound closure time, tensile strength of wound and regeneration of tissues at the wound site. Histological studies revealed evidences for the healing process by formation of fibrovascular tissue, epithelization and increased collagenization when compared to control.

Keywords – wound healing activity, *Plumbago rosea*, plumbagin, Plumbaginaceae

Introduction

Plumbago rosea (Plumbaginaceae) (Syn. *Plumbago indica* Linn.), known as senkodiveli in Tamil, grows as perennial herb in the plains of Bengal and South India (Anonymous, 1992). The plant is said to possess various medicinal properties in the indigenious system of medicine (Yoganarasimhan *et al.*, 2000). Plumbagin (**1**) (2-methyl, 5-hydroxy 1, 4-naphthoquinone) is the major constituent present in roots of *P. rosea*, and has been reported to possess various biological activities (Dhar and Rao, 1995).

Plumbago zeylanica another common species of the genus *Plumbago* is used in the Vietnamese traditional medicine for the treatment of wounds (Nguyen *et al.*, 2004). A preliminary study on the wound healing activity of *P. zeylanica* has been reported by (Suresh *et al.*, 2002). In the present communication we report the wound healing activity of chloroform extract of *P. rosea* and plumbagin in terms of wound contracting ability, wound closure time, tensile strength of wound, repair of tissues at the wound sites and histopathological features.

*Author for correspondence
Fax: +91-44-26207566; E-mail: chandbalki@yahoo.com

Experimental

Plant materials – The roots of *Plumbago rosea* were procured from local market in Chennai, Tamil Nadu, India and authenticated by identified by Dr. P. Brindha, Dept. of Botany, Captain Srinivasa Murti Drug Research Institute for Ayurveda, Arumbakkam, Chennai-600 106. A voucher specimen (No. 43) has been deposited in the herbarium of this institute.

Preparation of extract – The shade dried and coarsely powdered root (1 kg) was extracted with chloroform by cold percolation method (48 h). The $CHCl_3$ extract was filtered and distilled on a water bath. It was finally concentrated in vaccum to get a red brown syrupy mass (yield 8 g).

Isolation of plumbagin (1) – Plumbagin was isolated by stem distillation of the $CHCl_3$ extract of the roots (1 kg) (Saradha Vasanth *et al.*, 2004). The compound obtained as a reddish brown solid mass was crystallized from petroleum ether (yield 1.5 g, m.p. 78 °C). The identity was confirmed by comparison with an authentic sample (mp, mmp, co-TLC and superimposable IR).

Preparation of ointment – The wound healing activity of the chloroform extract and that of plumbagin were studied in yellow soft paraffin as the base (Carter, 1975). 100 g each of 0.5% (w/w) ointment of *P. rosea* extract

and 0.1% (w/w) of plumbagin in yellow soft paraffin were prepared.

Animals – Male Wistar albino rats (180 - 220 g) were obtained from the Tamil Nadu University of Veterinary and Animal Sciences, Chennai, Tamil Nadu, India. The animals were housed under standard laboratory conditions of 12 h light/dark cycle, 25 ± 2 °C with free access to a standard commercial pellet diet (manufactured by M/s Pranav Agro Industries Limited, Sangli, India) and water was provided *ad libitum*.

Models for wound healing activity – Wound healing activity was studied using two models *viz.* excision wound model and incision wound model.

Excision wound model – Four groups with six animals in each group were anaesthetized by an intraperitoneal injection of thiopentone sodium (3 mg/100 g body wt.). The mid dorsal region of each rat was shaved and an excision wound was made along the midline aseptically by cutting away a 500 mm^2 piece of skin from the shaved area (Morton and Malon, 1972). The wounds were of full thickness type, extending down to the subcutaneous tissue. Group I, untreated controls; Group II, topically treated with 0.5% w/w *P. rosea* extract ointment, Group III, topically treated with 0.1% w/w plumbagin ointment and Group IV, topically treated with reference standard framycetin sulphate cream (1% w/w). The application of the drugs was carried out daily till the wounds were completely healed. Wound contraction and wound closure time was monitored. Wound contraction was calculated as percentage reduction in wound area at 4-day intervals using the formula of Rashed *et al.* (2003). The progressive changes in wound area were monitored planimetrically by tracing the wound margin on graph paper every 4^{th} day.

Incision wound model – Four groups with six animals in each group were anaesthetized and a linear incision of 6 cm in length was at mid dorsal region which extended upto subcutis (Ehrlich and Hunt, 1969). Complete aseptic measures were taken and no local or systemic antimicrobials were used throughout the experiment. All the groups were treated in the same manner as mentioned in the excision wound model. After mopping the wound dry, the parted wound lips were apposed with interrupted sutures of 1 cm gap using surgical thread (Ethilon, No. 2-0) and a curved needle (No. 11). The rats were treated once daily for 10 days. The stitches were removed after 8 days and the tensile strength of the wound was measured on 10^{th} post wounding day (Lee, 1968).

Histopathological study was also carried out to evaluate the effect of *P. rosea* extract and plumbagin ointment on collagen formation.

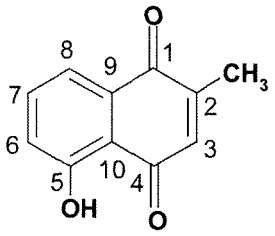

Fig. 1. Structure of plumbagin.

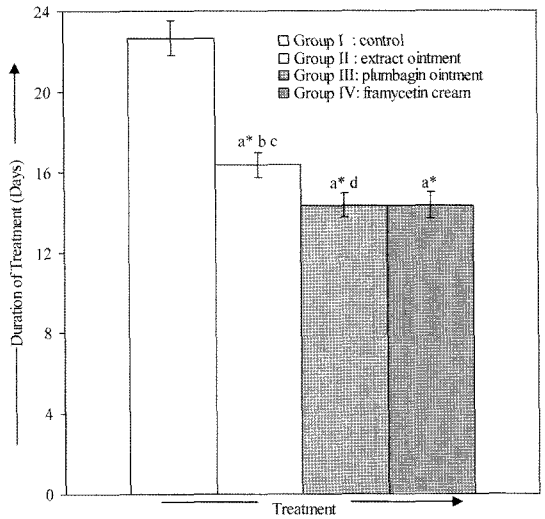

Fig. 2. Effect of *Plumbago rosea* extract, plumbagin and framycetin sulphate on wound healing day. a-as compared with group I; b-as compared with group III; c-as compared with group IV; d-as compared with group IV. Significance $*P < 0.001$.

Statistical analysis – The obtained data were statistically evaluated using ANOVA, expressed as mean ± SEM followed by Post Hoc Dunnett T3 multiple comparisons test using the 7.5 version of SPSS computer software. Results were considered significant at $P < 0.05$.

Results

In the present study, the progress of the wound healing induced by the *P. rosea* extract, plumbagin ointment and framycetin sulphate cream were studied by counting the number of days needed for wound contraction and complete epithelization and also by measuring the tensile strength of incision wound.

Calculation of wound contraction – The wound contraction on different days is shown in Fig. 2. The percentage of wound contraction was significantly greater in 0.1% w/w plumbagin ointment treated rats and complete healing was observed on 14th day indicating faster epithelization and collagenization when compared to control

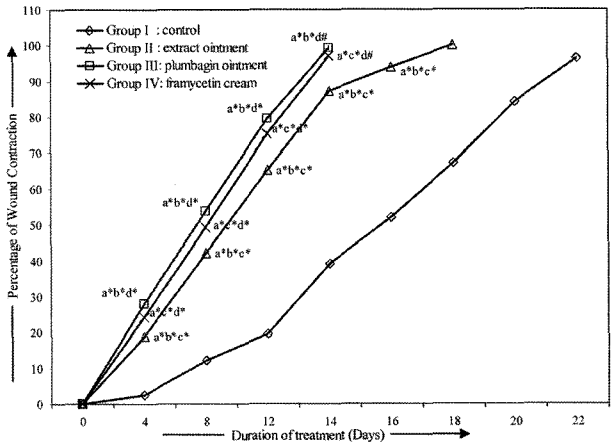

Fig. 3. Effect of *Plumbago rosea* extract, plumbagin and framycetin sulphate on wound healing by excision wound model in rats. a-as compared with group I; b-as compared with group III; c-as compared with group IV; d-as compared with group IV. Significance $*P < 0.001$, $\# P < 0.05$.

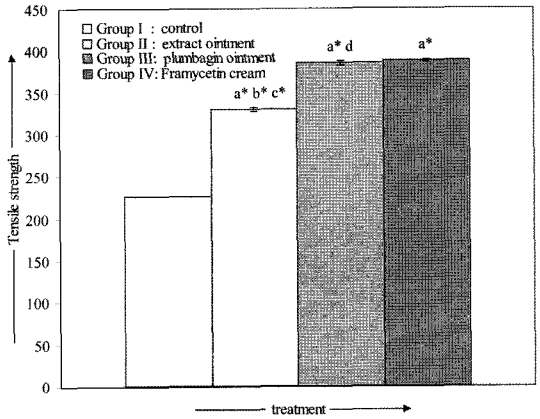

Fig. 4. Effect of *Plumbago rosea* extract, plumbagin and framycetin sulphate on wound healing by incision wound model in rats. a-as compared with group I; b-as compared with group III; c-as compared with group IV; d-as compared with group IV. Significance $*P < 0.001$.

(Fig. 3). It was observed that topical administration of 0.1% w/w plumbagin accelerated the progression of wound healing by 53.77 % on day 8 to 99.33% on day 14 which was slightly higher that those treated with reference standard drug which ranged from 49.37% to 97.06% activity respectively. The values shown by the *P. rosea* extract ointment were 42.13 and 87.16% respectively (Fig. 3). The time taken for wound closure with plumbagin ointment and framycetin sulphate treated group of animals (14 ± 2 days) was less than in the other groups. In case of the *P. rosea* extract ointment group it was 18 ± days whereas in control animals it took more than 26 days.

Tensile strength – In the incision wound studies, the tensile strength was significantly ($P < 0.001$) increased to 330.16, 385.16, 387.33 g after 10 days of topical treatment with *P. rosea* extract, plumbagin and framycetin-treated animals when compared to untreated group (226 g) (Fig. 4). The tensile strength of framycetin sulphate cream and plumbagin ointment treated groups were almost the same and were greater than *P. rosea* extract ointment treated group.

Histopathology – Control animals on the third day showed loss of superficial epidermis replaced by inflammatory exudates, consisting of RBC, neutrophils and a few lymphocytes. Dermis also showed scattered neutrophilic infiltration (Fig. 5A). Similarly on the fifteenth day in control animals the surface epidermis was still missing. The collagen deposits were moderate in the deep dermis and the upper dermis showed inflammatory infiltration. Superficial layer was covered by cell debris (Fig. 5B).

In *P. rosea* extract treated animals on the third day the upper epidermis was completely missing and covered by inflammatory exudates rich in neutrophils, macrophages and lymphocytes. The upper epidermis showed inflammatory exudates containing neutrophils and macrophages with oedema. Mild fibroblast activity was noticed (Fig. 5C). On the fifteenth day in *P. rosea* extract treated animals the wound defect was completely replaced by fibrovascular tissue with increased amount of collagen. Surface was yet to be covered by epidermis (Fig. 5D).

In the plumbagin treated animals of group III the superficial layer was necrotic with loss of epidermis, upper dermis oedematous with inflammatory cells, lower layer showing vascularisation (Fig. 5E). On the fifteenth day the defect was completely filled by fibrovascular tissue with high amount of collagen and the surface epithelium was yet to bridge the gap (Fig. 5F).

Discussion

Normal healing of wound involves the following stages. An initial inflammatory stage followed by fibrovascular tissue proliferation and secretion of mucopolysaccharides, formation of collagen fibres, shrinkage and drying of the scar. These are concurrent and independent stages. Hence drugs/factors that influence one stage need not affect the others (Udupa *et al.*, 1995a). The extracts of *Plumbago* sp. are reported to have anti-inflammatory and antiseptic activities (Gujar, 1990). The findings of the present study showed that the indigenous drug had a definite prohealing action. This is demonstrated by the increased percentage of wound contraction indicating faster epithelization and collagenization and gain in tensile strength, which indicate better maturation of collagen by increased cross-

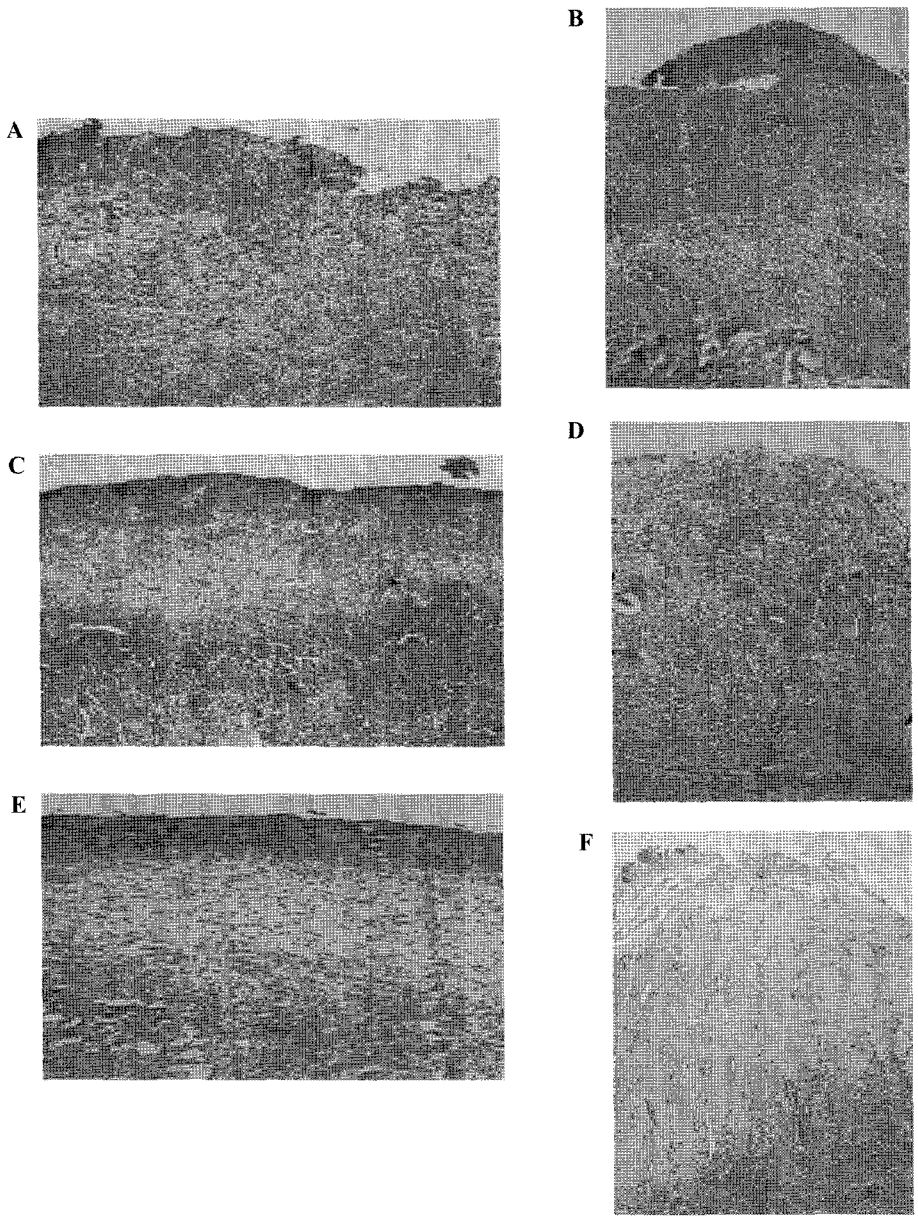

Fig. 5A-F. Skin plug of- (**A**) vehicle treated rat at 3-day post wounding; (**B**) vehicle treated rats at 15-day post wounding; (**C**) 3-day post wounding which received daily topical application of 0.5% (w/w) ointment of *Plumbago rosea* extract; (**D**) 15-day post wounding which received daily topical application of 0.5% (w/w) ointment of *Plumbago rosea* extract; (**E**) 3-day post wounding which received daily topical application of 0.1% (w/w) of plumbagin ointment; (**F**) 15-day post wounding which received daily topical application of 0.1% (w/w) of plumbagin ointment (H & E 80X).

linking. Results obtained in the present study suggest that treatment with 0.1% w/w plumbagin ointment in rats has hastened the healing process by increasing the rate of wound contraction and epithelization process. This is confirmed by increased healed area when compared to reference standard, extract and control.

Tensile strength was studied to support the wound healing activity of the plumbagin and *P. rosea* extract ointment. The increase in tensile strength of framycetin sulphate and plumbagin-treated groups was high which indicated higher collagen cross link formation and fiber strength (Udupa, 1994; Udupa *et al.*, 1995b). The fact that the increase in tensile strength can be directly related to collagen formation as shown in the case of *Aloe vera* (Chithra *et al.*, 1998) and *Centella asiatica* (Suguna *et al.*, 1996). The above findings suggest that both chloroform extract of *P. rosea* and plumbagin the major naphthoquinone present in the plant have significant wound healing

activity and was comparable to the standard drug framycetin sulphate cream, that was further supported by histopathological studies.

Acknowledgements

The authors wish to thank The Director, Central Council for Research in Ayurveda and Siddha, New Delhi for financial support.

References

Anonymous., The Wealth of India. Raw Materials. Vol. II, Publications and Information Directorate (CSIR), New Delhi, pp. 189-185 (1992).

Carter, S.J., Cooper and Gunn's Dispensing for Pharmaceutical Students, 12th ed. Pitman Medical, New York, pp. 200 (1975).

Chithra, P., Sajithlal, G.B. and Chandrakasan, G., Influence of *Aloe vera* on collagen characteristics in healing dermal wounds in rats, *Mol. Cell. Biochem.* **181**, 71-76 (1998).

Dhar, S.K. and Rao, P.G., Hormonal profile of plumbagin, *Fitoterapia* **66**, 442-446 (1995).

Ehrlich, H.P. and Hunt, T.K., Effect of cortisone and anabolic steroids on tensile strength of a healing wound, *Ann. Surg.* **170**, 203-206 (1969).

Gujar, G.T., Plumbagin, a natural occurring naphthoguinone. Its pharmacological and pesticidal activity, *Fitoterapia* **61**, 387-394 (1990).

Lee, K.H., Studies on the mechanism of action of salicylates. 3. Effect of vitamin A on wound healing retardation action of aspirin, *J. Pharm. Sci.* **57**, 1238-1240 (1968).

Morton, J.J. and Malone, M.H., Evaluation of vulneray activity by an open wound procedure in rats, *Arch. Int. Pharmacodyn. Ther.* **196**, 117-126 (1972).

Nguyen, A.T., Malonne, H., Duez, P., Vanhaelen-Fastre, R., Vanhaelen, M., and Fontaine, J., Cytotoxic constituents from *Plumbago zeylanica*, *Fitoterapia* **75**, 500-504 (2004).

Rashed, A.N., Afifi, F.U. and Disi, A.M., Simple evaluation of the wound healing activity of a crude extract of *Portulaca oleracea* L. (growing in Jordan) in *Mus musculus* JVI-1, *J. Ethnopharmacol.* **88**, 131-136 (2003).

Saradha Vasanth., Balakrishna, K., George, N. K. and Veluchamy, G., Synthesis of some plumbagin derivatives and their antibacterial activity, *J. Indian Chem. Soc.* **81**, 509-510 (2004).

Suguna, L., Sivakumar, P. and Chandrakasan, G., Effects of *Centella asiatica* extract on dermal wound healing in rats, *Indian J. Exp. Biol.* **34**, 1208-1211 (1996).

Suresh, R.J., Rajeswara Rao, P. and Mada, R.S., Wound healing effects of *Heliotropium indicum*, *Plumbago zeylanicum* and *Acalypha indica* in rats, *J. Ethnopharmacol.* **79**, 249-251 (2002).

Udupa, A.L., Kulkarni, D.R. and Udupa, S.L., Effect of *Tridax procumbens* extracts on wound healing, *Int. J. pharmacol.* **33**, 37-40 (1995a).

Udupa, S.L., Inhibition of lysyl oxidase by isoniazid and its effect on wound healing, *Indian J. Exp. Biol.* **33**, 278-280 (1995b).

Udupa, S.L., Udupa, A.L. and Kulkarni, D.R., Studies on the anti-inflammatory and wound healing properties of *Moringa oleifera* and *Aegle marmelos*, *Fitoterapia* **65**, 119-123 (1994).

Yoganarasimhan, S.N. and Chelladurai, V., Medicinal plants of India. Vol 2, published by Yoganarasimhan SN, Cyber Media, Bangalore, India, pp. 425 (2000).

(Accepted March 8, 2006)