



Wound Healing Activity of *Annona muricata* extract

Padmaa M Paarakh^{1,*}, Chansouria JPN² and Khosa RL³

^{1,*} Department of Pharmacognosy, The Oxford College of Pharmacy, J P Nagar, I. Phase, Bangalore 560 078, India

² Centre for Experimental Medicine and Surgery, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India

³ Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi 221005, India

For correspondence: Dr. Padmaa M Paarakh, Principal and HOD, Department of Pharmacognosy, The Oxford College of Pharmacy, J.P. Nagar, I. Phase, Bangalore 560078

E-mail: padmaparas@hotmail.com

Received on: 12-10-2008; Accepted on : 09-01-2009

ABSTRACT

The wound healing activity of alcoholic extract of stem bark of *Annona muricata* was evaluated in albino rats by open wound method for a period of 12 days. Extract showed marked reduction in area of the wound in comparison with that of the control group from 4th days onwards suggesting its possible use in healing the wound.

Key words : Wound healing; *Annona muricata* stem bark; open wound method

INTRODUCTION

Wounds were probably the first medical problem faced by the human race. However it is interesting to note that the knowledge of wounds and their management remained in primitive and stunted stage. Although major improvements in almost all branches of surgery have occurred, progress in improving wound healing per se has been very slight. In Ayurveda, the types of wounds and their management (about sixty procedures) has been elaborated in greater details by the pioneer ancient surgeon Sushruta (Deshpande et al., 1970). All studies were centered mainly on the prevention of deficiencies of Vitamin, trace elements and proteins that delay wound healing. It was only with the advent of growth factors that the normal healing could be enhanced by therapeutic manipulation took birth. From the past few centuries, there has been growing interest in using number of herbal drugs for the management of wound singly or in combination in different forms such as juice, decoction, and ointment in different vehicles.

Therefore, in this context, *Annona muricata* plant was selected for studying the wound healing activity, as it finds a variety of medicinal uses in Traditional System of Medicine including Kidney stones, fever, nervousness, ulcers and wounds (Wealth of India, 1985). The plant has been reported to possess acetogenins as major phytoconstituents (Padma et al., 1996) which are responsible for number of activities such as antitumor, immunomodulator, antispasmodic, antimalarial, pesticidal, antiparasitic, antibacterial, antifungal and antihelmintic activity (Padma, 1997). Therefore in the present study, the alcoholic extract of stem bark of *Annona muricata* which is rich in acetogenin is taken to evaluate the wound healing activity as there is no scientific data available

to prove the traditionally mentioned uses of healing wound.

Experimental

Plant Material and Extraction

The stem bark of *Annona muricata* (Annonaceae) Linn were obtained from Tirunelveli district (Tamilnadu, India) and authenticated by Dr. V. Chelladurai, Survey of Medicinal Plant Unit, Government Siddha Medical College, Tirunelveli. A voucher specimen PP/PH/01/95 is preserved at the Department of Pharmaceutics, Banaras Hindu University. The plant material was defatted with petroleum ether (60^o – 80^oC) and extracted with ethanol (95 %) by soxhlation. The ethanol extract was dried and made into ointment of 4 % w/w using simple ointment as the base. Simple ointment base was prepared as per IP (Indian Pharmacopoeia, 1966).

Experimental Animals

Fourteen adult healthy albino rats of Charles-Foster strain (200 ± 20 g, b.w.) were housed in standard conditions (temperature of 25 ± 2^o C with 55 % – 65 % relative humidity and a 12-h light dark cycle). They had free access to water and normal laboratory diet (Lipton India Ltd). They were acclimatized to animal house condition for 2 weeks before starting the experiment.

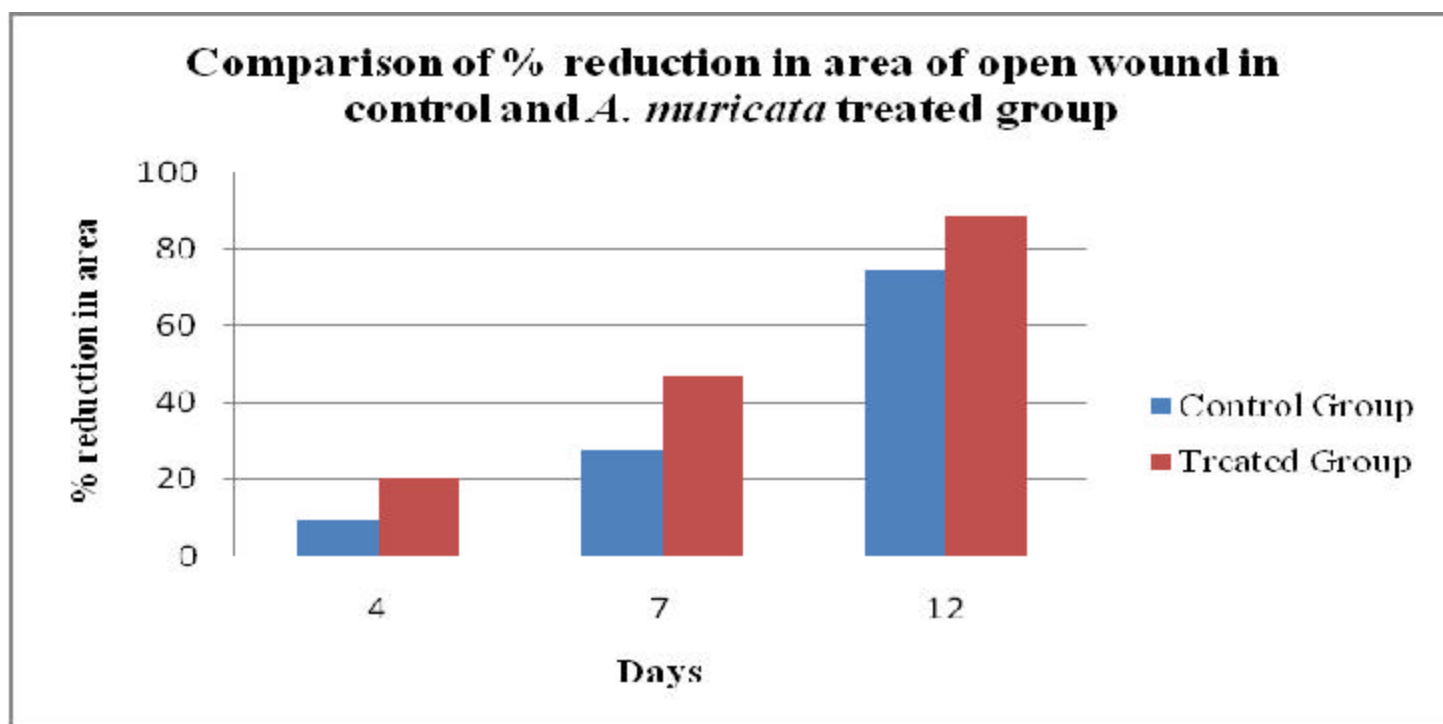
Wound healing activity by open wound method

The animals were anaesthetized by giving ketamine hydrochloride at a dose of 120 mg/kg intravenously. Following anesthesia, the abdomen of each rat was shaved and prepared for operation. A cutaneous wound of 4 sq cm area of skin was made on the back of each rat under aseptic condition and animals were kept as such individually in separate cages (Morton, 1972; Nayak et al., 2007). Seven animals

Table 1: Effect of *A. muricata* extract on open wound. Values are expressed in mean \pm SEM

Day	Area in sq cm	
	Control group [n=7]	Treated group [n=7]
1 st day	9.21 \pm 0.75	9.98 \pm 0.35
4 th day	8.36 \pm 0.68	8.01 \pm 0.65 ^a
7 th day	6.68 \pm 0.51	5.28 \pm 0.39 ^b
12 th day	2.35 \pm 0.47	1.14 \pm 0.13 ^a

^a P < 0.05 compared with control group; ^b P < 0.001 compared with control group.

Figure 1: Comparison of % reduction in area of open wound in control and *A. muricata* treated group

each served as control and treated group; simple ointment and ointment containing the extract was applied everyday topically for a period of 12 days respectively. Then measure the area of the wound by mapping the wounded area on a tracing paper quantitatively and then estimating the actual cross sectional area with help of planimetry. Results are expressed in a sq cm. The rate of wound contraction was measured in different treated and control groups on 0 day, 4th day, 7th day and 12th day.

Statistical analysis

Data were statistically analyzed using Student's *t*- test.

Results and Discussion

The area of wound healing in sq cm is given in the Table no.1 and the percentage reduction in the area of the wound is given in the Fig. no. 1.

A wound which is disrupted state of tissue caused by physical, chemical, microbial or immunological insult ultimately heals either by regeneration or fibroplasias. Healing progresses

through three general stages (1) inflammatory, (2) proliferative & repair and (3) remodeling stages (Schilling, 1968). During inflammatory stages, as a result of injury, the blood circulation in the local area are reduced which leads to local hypoxia, acidosis and low pH (Udupa, 1996). Following the inflammatory response to injury or wound, a tremendous proliferation of cells takes place which is actually responsible for the process of repair. The cellular responses include blood supply, surface covering and reproduction of collagen which ultimately helps to bind the wound margin and development of permanent functional tensile strength (Hunt, 1964).

In the healing of the excised wounds, the mechanism of wound contraction plays an important role. The term contraction implies reduction in the wound size and is believed to take place as a result of movement of wound edges towards the centre. Generally the wound contraction continues for 10-15 days and therefore it stops. It has been shown that circular wound contract at a relatively slower rate than the square/

rectangular ones (Billingham et al., 1956). Most studies of the wound healing were made on excised surface wound and were concerned with the rate and extent of epithelization. In large open wounds, it is recognized that proper debridement of dead and devitalized tissue was essential for prompt healing.

From the Table no. 1, it is clear that a 4 % alcoholic extract of *Annona muricata* as an ointment applied daily for a period of 12 days significantly reduced the area of open wounds as compared with those of control group. The actual healing seems to proceed from the 4th day onwards from the day of wounding. The observation revealed a better healing pattern as percentage reduction in the wound area of the treated group (19.74, 47.09 and 88.58%) than that of the control group (9.22, 27.47 and 74.47%) on 4th, 7th and 12th day respectively. The result obviously indicates the wound healing property of *Annona muricata* extract.

Acknowledgement

The authors gratefully acknowledge the financial assistance from University Grants Commission, India.

REFERENCES

1. Anonymous, Indian Pharmacopoeia, Vol 1, Ministry of Health and Family Welfare, Govt. of India, New Delhi, 1966, 231-233.
2. Anonymous, The Wealth of India, (Revised) Raw Materials, Vol. IA, Publication & Information Directorate, CSIR, New Delhi, 1985, 278-281.
3. Billingham RE, Russel PS, Studies on wound healing with special reference to the phenomenon of contracture in experimental wounds in rabbit's skin, *Ann Surg*, 144, 1956, 961-981.
4. Deshpande PJ, Pathak SN, Gode JD, *Advance in Research in Indian Medicine*, BHU Press, Varanasi, 1970, 269-280.
5. Hunt TK, A new method of determining tissue oxygen tension, *Lancet*, 2, 1964, 1370-71.
6. Morton JJ, Malone MH, Evaluation of vulnerary activity by an open wound procedure in rats, *Arch Int Pharmacodyn*, 196, 1972, 117-26.
7. Nayak BS, Sandiford S, Maxwell A, Evaluation of the wound healing activity of ethanolic extract of *Morinda citrifolia* leaf, *Evidence based Comple and Alternate Medicine*, 78, 2007, 1-6.
8. Padma P, Khosa RL, Sahai M, Acetogenin from Genus *Annona* – A Review, *Indian Journal of Natural Products*, 12(3), 1996, 3-21.
9. Padma P, *Phytochemical studies and Evaluation of Biological activity of some Medicinal Plants*, PhD Thesis, Department of Pharmaceutics, Banaras Hindu University, Varanasi, 1997, 141-195.
10. Schilling JA, Wound healing, *Physio Review*, 48, 1968, 374-423.
11. Udupa SL, *International Seminar on Free Radicals mediated disease and Ayurveda*, BHU, September 2-4, 1996, Abstract A-9.

Source of support: University Grants Commission, India; Conflict of interest: None Declared