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Evaluation of wound healing activity of *Ichnocarpus frutescens* root

A.Pandurangan [a*], R.L Khosa [a] and S.Hemalatha [b]

aSchool of Pharmacy, Bharat Institute of Technology, Bypass Road, Partapur, Meerut – 250103, Uttar Pradesh, India.
[b]Department of Pharmaceutics, Banaras Hindu University, Varanasi, India

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Abstract

The methanol extract of Ichnocarpus frutescens R. Br (Apocynaceae) roots were investigated for the evaluation of their wound healing potential on different experimental models of wounds in rats. The methanol extract of roots (MEIF), in the form of an ointment with two different concentrations (1 % and 2 % w/w ointment of root extract in simple ointment base) was evaluated for wound healing potential in an excision wound model and an incision wound model in rats. Both concentrations of the methanol extract ointment showed significant responses in both the wound types tested when compared with the control group. The effect produced by the extract ointment, in terms of wound contracting ability, wound closure time, regeneration of tissues at wound site, tensile strength of the wound and histopathological characteristics were comparable to those of a standard drug framycetin sulphate cream.

Keywords: Ichnocarpus *frutescens*; Wound healing; Excision wound; Incision wound

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INTRODUCTION

Research on wound healing agents is one of the developing areas in modern biomedical sciences. Many traditional practitioners across the world particularly in countries like India and China with age old traditional practices have valuable information of many lesser-known hitherto unknown wild plants used by the traditional healers for treating wounds and burns. Several drugs of plant, mineral and animal origin are described in the traditional texts of Indian systems of medicine like Ayurveda for their healing properties under the term 'Vranaropaka'. Besides the classical systems of Indian Medicine, the folk and the tribal medicine also employ a number of plants and animal products for treatment of cuts, wounds and burns.

Ichnocarpus frutescens R. Br (Apocynaceae), commonly known as siamlata, is an evergreen, laticiferous, woody creeper with rusty red appearance, found almost throughout India. *Ichnocarpus frutescens* is used in the indigenous system of medicine in the treatment of fevers, gout, rheumatism, arthritis, epilepsy, venereal diseases, herpes and skin diseases [1, 3, 5]. Studies on chemical constituents of the plant revealed the presence of ursolic acid and kaempferol in the leaves [4], lupeol, fridelin, β-sitosterol from stems [5]. Pharmacological investigations have demonstrated that *I. frutescense* possess antioxidant activity and anti-inflammatory activity [6]. The aim of this study was to evaluate the wound healing activities of methanolic extract of *Ichnocarpus frutescens* (MEIF).

MATERIALS AND METHODS

Plant material

The roots of *Ichnocarpus frutescens* R. Br were collected during the month of June 2005 from Chennai, Tamilnadu, India. The plant material was taxonomically Identified and authenticated by Prof. P. Jayaraman, Taxonomist, Plant Anatomy Research Centre, Chennai, India. A voucher specimen (PARC/24/06) has been deposited in the Herbarium of the Department of Pharmaceutical Technology, Meerut Institute of Engineering and Technology, India, for future reference.

Extraction procedure

The powdered plant material was extracted using 95 % methanol and the solvent was completely removed by vacuum distillation to yield a residue 20.7 %, w/w respectively. This extract (MEIF) was examined chemically and was observed to contain flavonoids, terpenoids, and sterols. These constituents were confirmed using thin-layer chromatography (TLC).The extract was stored in a refrigerator and a weighed amount of MEIF was suspended in 1 % simple ointment and used for the present study.

Animals

Albino (Wister) rats 180-200 g of either sex were used. The animals were kept in the standard polypropylene cages and provided with food and water *ad libitum*. The animals were acclimatized for period of 14 days prior to performing the experiments. The experimental protocols were approved by Institutional Animal Ethics Committee (Regn No: 711/02/A/CPESEA).

Excision wound model

Four groups of animals containing six in each group were anaesthetized by open mask method with anaesthetic ether. The rats were depilated on the back and a predetermined area of 500 mm^2 full thickness skin was excised in the dorsal interscapular region [7]. The animals of group I were left untreated and considered as the control, group II served as reference standard and treated with 1 % w/w framycetin sulphate cream (FSC), animals of group III and IV were treated with 50 mg of ointment prepared from methanolic extract of *Ichnocarpus frutescens* ((MEIF 1 % and 2 %w/w). The progressive changes in wound area were monitored planimetrically by tracing the wound margin on a graph paper every alternate day. The change in healing of wound, i.e. the measurement of wound area on graph paper was expressed as unit (mm^2). Wound contraction was expressed as percentage reduction of original wound size.

Histopathological examinations

A specimen sample of skin tissues of each group of rats were taken out from the healed wounds of the animals in the above excision wound model for histopathological examinations. The thin sections of the tissues were stained with Eosin I bluish solution and observed for the histological changes under microscope [8].

Incision wound model

Four groups of animals containing six in each group were taken. The animals were anaesthetized under light ether anaesthesia. One full thickness Para vertebral incision of 6 cm length was made including the cutaneous muscles of the depilated back of each rat. Full septic measures were not taken and no local or systemic antimicrobials were used through out the experiment. After the incision was made, the parted skin was kept together and stitched with sutures, 1 cm apart [7]. The continuous threads on both wound edges were tightened for good adoptation of wound and it was left undressed. The ointment of the root extract (MEIF 1 % and 2 %w/w), standard drug (nitrofurazone ointment) and simple ointment were applied to the wound twice daily, until complete recovery to the respective groups of animals.

RESULTS

Excision wound study

The progress of the wound healing induced by *I. frutescens* root extract ointments ((1 % and 2 %w/w) treated groups, simple ointment (control) treated group and framycetin sulphate cream (standard drug) treated group of animals are shown in Table 1. It is observed that the wound contracting ability of the extract ointment in different concentrations was significantly greater than that of the control (i.e. simple ointment treated group). The MEIF 1 % (w/w) extract ointment treated groups showed significant wound healing from the fourth day onwards, which was comparable to that of the standard drug, i.e. framycetin sulphate cream treated group of animals. The wound closure time was lesser, as well as the percentage of wound contraction was much more with the 2 % w/w treated group (18 days for 100% contraction which was almost similar to that of the framycetin sulphate cream treated group). MEIF (2 % w/w) ointment treated group of animals showed significant wound contraction from the sixth day onwards and achieved 100% with the wound closure time of 19 days.

Incision wound study

The measurement of the effect of the extract and standard drug on the tensile strength of the incision wound is shown in Table 2. The tensile strength of the MEIF (1 % and 2 % w/w), extract treated group and the framycetin sulphate cream treated group were comparable to each other.. Thus both concentrations of the extract as well as the standard drug showed a significant increase in tensile strength in the 10 days old wound.

Histopathological evaluation

The multiple sections studied in histopathological examination of the tissues of the wound area treated with the extract ointments (MEIF 1 % and 2 %w/w), 1 % w/w framycetin sulphate cream, and simple ointment (control) treated groups. The histological examination showed that the original tissue regeneration was much greater in the skin wound treated with extract ointments and framycetin sulphate cream treated group without any oedema, congestion or inflammatory

changes. More relative fibrosis were observed in the framycetin sulphate cream treated wound with flattened rete ridges in the epidermis comparing to the skin wound treated with either 1 % or 2 % of the extract ointment.

Estimation of Hydroxyproline

Throughout the course of healing, hydroxyproline was found to be more in all treated group than control, which are important constituent of extra cellular matrix for healing. This biochemical contents were more in 2 % (w/w) MEIF than 1 % (w/w) MEIF ointment.

DISCUSSION

Wound healing is the physiological response to the tissue injury that results in the replacement of destroyed tissue by living tissue and thus restoration of tissue integrity. The mechanism of wound repair occurs by four basic processes such as inflammation, wound contraction, epithelialisation and granulation tissue formation. Inflammation starts immediately after the disruption of tissue integrity. The platelets became adherent with clotting factors and form haemostatic plug to stop bleeding from the vessels. The prostaglandins (PGE1 and PGE2) are released in the inflammation area and seem to be the final mediators of acute inflammation and may play a haemostatic role for white cells and fibroblasts. The active motile white cells migrate into the wound and start engulfing cellular debris, at the initial stages wound contraction begin slowly but became rapid after 3 or 4 days. The myofibroblsts present in the margin of the wound appear to constitute the machinery for the wound contraction. Theses are responsible for overlaying debris. The epithelialisation of the wound mainly occurs by proliferation and migration of the marginal basal cells lying close to the wound margin. The hematoma within the wound may be replaced by granulation tissue, which consists of new capillaries and fibroblasts. The fibroblasts are responsible for production of the mucopolysaccharide ground substance. The lymphatics develop new nerve fibers and there is also formation of scar tissue in which collagen turn over increases.

Preliminary phytochemical analysis revealed the presence of flavonoids, terpenoids and steroids. Flavonoids are known to reduce lipid peroxidation by preventing /slowing onset of cell necrosis as well by improving vascularity and drugs that inhibits lipid peroxdiation is also belived to increase viability of collagen fibers by increasing the strength of collagen fibers, the circulation, preventing the cell damage and by promoting DNA synthesis [9]. Flavonoids [10] and terpenoids [11] have been reported to promote wound healing due to its astringent and antimicrobial property, which may be contributing to wound contraction and increase rate of epithelialisation. Antioxidant property of flavanoids and terpenoids may also be contributing to wound healing [12]. The wound healing potential of the *I. frutescens* extract may probably be as a result of the presence of a mixture of phytoconstituents including flavonoids, terpenoids, steroids, etc., the isolation of which is under way in our laboratory. Thus from this study it is concluded that the *I. frutescens* root extract has a reproducible wound healing potential and thereby justifies its use in folklore medicine in India.

Table17.Evaluation of MEIF extracts on wound healing by excision wound method in rats.

Group dose	0th Day	2nd Day	4th Day	8th Day	12th Day	16th Day	18th Day	20th Day
Control (1% Simple ointment)	502.7 ± 3.29	456.7± 1.68 (9.15%)	384.3 ±3.32 (23.54%)	302.7 ±2.40 (39.78%)	236.3 ±2.27 (52.99%)	133.7 ±2.65 (73.40%)	68.33 ±1.58 (86.40%)	24.67 ±1.52 (95.09%)
1 % w/w framycetin sulphate cream	502.7± 2.108	445.7± 4.88 (11.33%)	323.7± 3.32 (35.60%)	172.3± 3.63 (65.72%)	102.3± 2.75 (79.64%)	19.33± 1.43 (96.15%)	-- [100%)	- [100%)
MEIF (1% ointment)	503.0 ± 2.51	448.2±2.19 (10.89%)	345.0±3.29 (31.41%)	208.3±4.04 (58.58%)	120.3±3.20 (76.08%)	21.33±1.76 (95.75%)	- (100 %)	-- [100%)
MEIF (2% ointment)	502.3 ± 3.70	447.7±4.77 (10.86%)	367.3±3.52 (26.87%)	219.5±2.55 (56.30%)	135.0±2.29 (73.12%)	75.33±2.56 (85.00%)	9.0±1.52 (98.20%)	- (100%)

Wound area (mm^2) mean ± SE and percentage of wound contraction

Table 2.Evaluation of MEIF extracts and standard drug on incision wound model in rats

Treatment	Excision wound Tensile strength (g) (mean ± SE)	Incision wound Epithelialisation period (days)
Control (1% Simple ointment)	417.6 ± 1.63	24.2 ±1.28
1 % w/w framycetin sulphate cream	547.4 ± 1.88**	17.8 ± 0.88**
MEIF (1% ointment)	495.8 ± 3.77**	16.1 ± 0.92**
MEIF (2 % ointment)	528 ± 1.41**	17.6 ± 1.48**

All values are represented as mean ± S.E.M. (n = 6). ANOVA followed by Dunnett's't' test. *$P < 0.05$, **$P < 0.01$ when compared to control.

Table 3. Hydroxyproline content

Serial no.	Treatment	Hydroxyproline (mg/g of tissue)		
		4	8	12
1	Simple ointment	21.2 ± 1.69	32.7 ± 1.15	38.0 ± 2.70
2	1 % w/w Framycetin sulphate cream	42.2 ± 1.24*	57.36 ± 1.06*	81.3 ± 2.21*
3	MEIF (1 % ointment)	30.1 ± 1.28*	46.8 ± 1.89*	60.5 ± 1.58*
4	MEIF (2 % ointment)	39.4 ± 1.18*	53.4 ± 1.65*	76.1 ± 1.50*

Values are expressed in mean ± S.E.M. from five rats. *p < 0.001 statistically significant difference in comparison with control group.

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