Wound Healing Activity of *Leucas hirta*

B. K. MANJUNATHA*, S. M. VIDYA[1], V. KRISHNA[2] AND K. L. MANKANI[3]

Department of Botany and [1]Biotechnology, S. R. N. M. N. College of Applied Sciences, Shimoga-577 201, [2]Department of Biotechnology, Kuvempu University, Shankaraghatta-577 451, [3]Department of Pharmacology, National College of Pharmacy, Shimoga-577 201, India.

The wound healing efficacy of aqueous and methanolic leaf extracts of *Leucas hirta* was evaluated in excision, incision and dead space wound models. The parameters studied include rate of wound contraction, period of complete epithelialization, tensile strength of incision wound and granulation tissue, granulation tissue dry weight, hydroxyproline content and histological studies of granulation tissue. Among the two extracts studied, methanol leaf extract was found to possess significant wound healing activity followed by aqueous extract, which was evidenced by decrease in the period of epithelialization, increase in the rate of wound contraction, skin breaking strength, granulation tissue dry weight, hydroxyproline content and breaking strength of granulation tissue. Histopathological study of the granulation tissue evidenced increased collagenation when compared to control group of animals.

The plant *Leucas hirta* (Roth) Spreng., (Labiateae) is a herb or undershrub, sparsely distributed in Deccan Peninsula and Western Ghats[1]. The plant is under threat because of the anthropological activities and overexploitation of this herb in and around the forest areas by the medical practitioners. The tribal groups of the Western Ghats use leaves as antiseptic, wound healer in septic wounds, in treating fever, cough, snake bite, and liver disorders[2]. Review of the literature revealed that though this plant is known for several pharmacological activities by the tribal groups of the Western Ghat region, it has not been subjected to scientific evaluation. Hence an attempt has been made to evaluate the wound healing property of the plant.

***For correspondence**

E-mail: doctor_bkm@yahoo.com

Leaves of *Leucas hirta* were collected from the Kuduremukha reserve forest of Chikkamagalur District, Karnataka state, during December 2003 and identified by the first author. Taxonomic authenticity was confirmed by referring to herbarium specimen at Madras Herbarium, Botanical Survey of India, Southern Circle, Coimbatore, and a voucher specimen (BKM-234) is deposited in the departmental herbaria, Department of Biotechnology, Kuvempu University, Shankaraghatta, as authentic specimen for future reference. The leaves were shade-dried for a week, powdered mechanically (sieve no. 10/44), and stored in airtight containers. About 250 g of the powdered material was subjected to Soxhlation and exhaustively extracted with 70% methanol for 48 h. The

solvent was distilled off at low temperature under reduced pressure using rotary flash evaporator (Buchi, Flawil, Switzerland). The yield was 30.8% w/w. Another 250 g of the powdered material was boiled in distilled water for 30 min, kept for 3 d with intermittent shaking, filtered and concentrated using rotary flash evaporator to get the aqueous extract. The yield was 20.2% w/w. Both the extracts were dried in dessicator and subjected to preliminary phytochemical tests[3]. Two types of drug formulations were prepared from each of the extracts. For topical administration, 5% w/w ointment gel was prepared in 2% sodium alginate. For oral administration, suspensions of 35 mg/ml of aqueous and methanol leaf extracts were prepared in 1% gum tragacanth.

Wistar rats of either sex weighing 150-200 g were procured from the National College of Pharmacy, Shimoga, and were maintained at standard housing conditions. The animals were fed with commercial diet (Hindustan Lever Ltd., Bangalore) and water *ad libitum* during the experiment. The study was permitted by the Institutional Animal Ethical Committee, (Reg. No.144/1999/CPCSEA/SMG). Acute toxicity study was conducted for both the extracts by staircase method[4]. The LD_{50} of aqueous and methanol leaf extracts were found to be 350 mg/kg. One-tenth of the dose of the extracts (35 mg/kg) was selected for the evaluation of wound healing activity[5]. Excision, incision, and dead space wound models were used to evaluate wound healing activity. The rats were inflicted with excision wounds under light ether anaesthesia[6]. A circular wound of about 500 mm^2 was made on depilated ethanol-sterilized dorsal thoracic region of rats. The animals were divided into four groups of six each. The group I was considered as the control, the group II served as reference standard and treated with 1% w/w framycetin sulphate cream (FSC), the group III and IV animals were treated with 50 mg of ointment gel prepared from aqueous and methanol leaf extracts of *Leucas hirta*, respectively. The ointment gel was topically applied once in a day, till the epithelialization was complete, starting from the day of the operation. The parameters studied were wound closure and epithelialization time. The wounds were traced on mm^2 graph paper on d 4, 8, 12, 16, and 18 and thereafter on alternate days until healing was complete. Percent wound closure and the period of epithelialization was calculated. In incision wound model, 6 cm long paravertebral incisions were made through full thickness of the skin on either side of the vertebral column of the rat[7]. The wounds were closed with interrupted sutures 1 cm apart. The animals were divided into four groups of six animals each. The grouping of experimental animals was similar to that of excision wound model, and the ointment gel containing 5% w/w of aqueous and methanolic leaf extracts in 2% sodium alginate was applied topically once in a day. The sutures were removed on day 8 after inflicting wound. The skin breaking strength of the wounds was measured on day 10 following continuous water flow technique[8]. In dead space wound model, the animals were divided into three groups – six rats in each group. The group I served as control, which received 1 ml of 1% gum tragacanth/kg. The animals of group II and III received oral suspensions of aqueous and methanol leaf extracts in the dose of 35 mg/kg p.o., respectively. Under light ether anaesthesia, dead space wounds were created by subcutaneous implantation of sterilized cylindrical grass piths (2.5×0.3 cm), one on either side of the dorsal paravertebral surface of rat[9]. The granulation

TABLE 1: EFFECT OF TOPICAL APPLICATION OF AQUEOUS AND METHANOL LEAF EXTRACTS OF *LEUCAS HIRTA* ON EXCISION WOUND MODELS

Post wounding days	Wounding area (mm^2)				ANOVA	
	Control	FSC1% w/w	Aqueous leaf extract 5% w/w	Methanol leaf extract 5% w/w	F	df
0	512.29±0.52 (0.00)	502.68±0.48 (0.00)	510.52±0.63 (0.00)	515.57±0.60 (0.00)	95.2	3,20
4	468.48±0.67 (8.55)	328.43±0.57* (34.66)	362.54±0.46* (28.98)	320.41±0.58* (37.85)	1.392	3,20
8	368.67±0.58 (28.03)	108.37±0.54* (78.44)	172.39±0.64* (66.23)	156.64±0.60* (69.61)	3.779	3,20
12	265.55±0.47 (48.16)	46.32±0.55* (90.78)	110.32±0.55* (78.39)	98.35±0.56* (80.92)	3.125	3,20
16	168.16±0.65 (67.17)	0.00* (100)	18.11±0.61* (96.45)	10.51±0.63* (97.96)	2.142	3,20
18	132.57±0.54 (74.12)	0.00* (100)	8.31±0.49* (98.37)	0.00* (100)	3.152	3,20
Period of epithelialization	23.48±0.13	16.40±0.15*	18.86±0.11*	17.56±0.18*	618.2	3,20

N=6 animals in each group.*P<0.01 indicates 'significant' compared to control. Values are expressed as mean±SE. figures in parenthesis indicate percentage of wound contraction

tissues formed on the grass piths were excised on day 10 after inflicting wound. The dry weight of the granulation tissue and the breaking strength was measured. Simultaneously, granulation tissue so harvested was subjected to hydroxyproline estimation[10] and histopathological study to evaluate the effect of the extracts on collagen formation. The data were subjected to ANOVA followed by Tukey's multiple comparison test, and the values of $P \leq 0.01$ were considered statistically significant.

The preliminary phytochemical tests of leaf extracts revealed the presence of flavonoids, alkaloids, tannins, saponins, glycosides, steroids, and triterpenoids. Effect of aqueous and methanol leaf extracts of *Leucas hirta* on excision wound model is presented in the Table 1. The animals of group I showed complete epithelialization on 23.5±0.13 post wound day, whereas group II animals treated with standard drug FSC showed complete epithelialization on 16.4±0.15 post wound day. Compared to control group, the animal groups treated with aqueous and methanol leaf extract showed decrease in the period of complete epithelialization (18.9±0.11; 17.6±0.18, respectively). Percentage closure of wound area was significantly high in methanol leaf extract treated group followed by aqueous extract treated group of animals. The rate of wound contraction was less in control group of animals, whereas the percentage of wound closure was high in methanol leaf extract treated group followed by

TABLE 2: EFFECT OF AQUEOUS AND METHANOL LEAF EXTRACTS OF *LEUCAS HIRTA* ON INCISION WOUND MODELS

Group (N)	Tissue breaking strength (g)
Control	323.03±2.66
Framycetin sulphate cream	588.79±3.31*
Aqueous leaf extract	483.69±2.80*
Methanol leaf extract	560.67±3.60*
ANOVA	
F	1462.0
df	3,20

N=6 animals in each group.*$P \leq 0.01$ indicates 'significant' when compared to control. Values are expressed as mean±SE

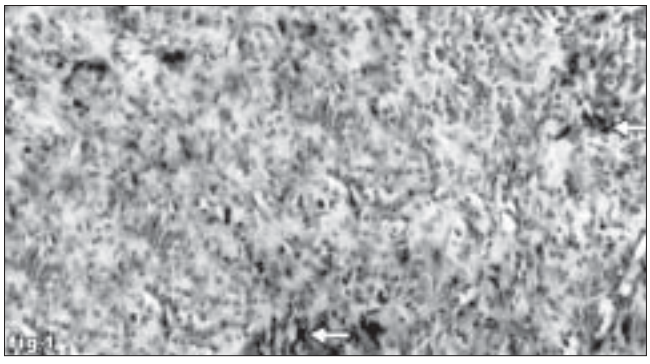

Fig. 1: Histology of granulation tissue of control group of animals.

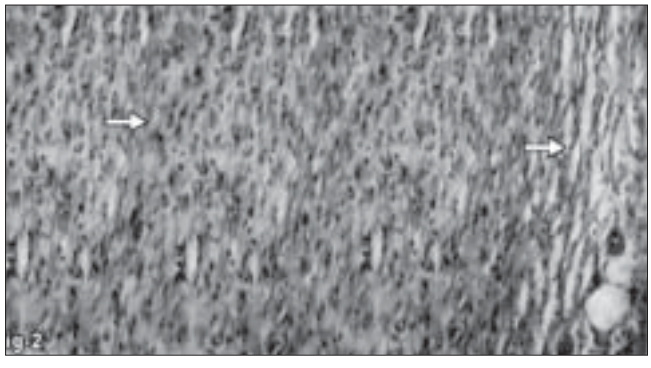

Fig. 2: Histological section of granulation tissue of the aqueous leaf extract treated animal showing moderate collagen deposition.

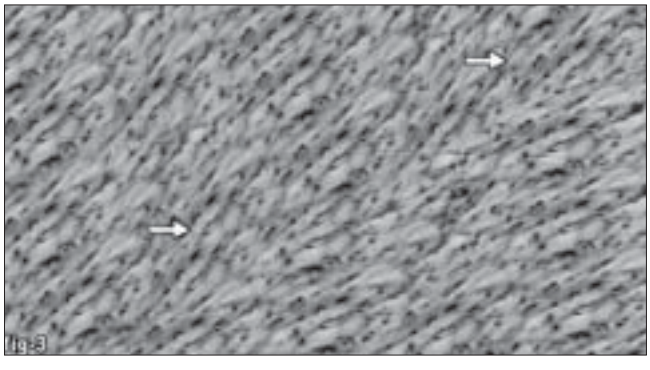

Fig. 3: Histological section of granulation tissue of the animal treated with methanol leaf extract showing increased collagenation.
The sections were stained with haematoxylin and eosin. They were observed under the magnification of 100x. Arrows in the figures indicate the collagen fibres.

TABLE 3: EFFECT OF AQUEOUS AND METHANOL LEAF EXTRACTS OF *LEUCAS HIRTA* ON DEAD SPACE WOUND MODELS

Groups (N)	Granulation tissue dry weight (mg/100 g)	Breaking strength (g)	Hydroxyproline (µg/100 mg)
Control	35.17±0.58	246.65±3.55	1386.54±0.46
Aqueous leaf extract	52.52±0.65*	357.00±2.22*	1980.58±0.64*
Methanol leaf extract	60.35±0.54*	396.33±2.30*	2268.16±0.68*
ANOVA			
F	470.5	791.7	5.615
df	2,15	2,15	2,15

N=6 animals in each group.*$P<0.01$ indicates 'significant' compared to control. Values are expressed as mean±SE. In excision wound model, figures in parenthesis indicate percentage of wound contraction

aqueous leaf extract treated animal group, indicating the effect of the plant on promoting healing of excision wound. In incision wound model, significant increase in the skin breaking strength was observed in methanol leaf extract treated groups of animals (561±3.6) followed by aqueous extract treated group of animals (484±2.8), indicating the effect of *L. hirta* leaf extract in maturation of collagen fibres (Table 2). Dead space wound model was used to study the effect of the extracts on granulation and collagenation of the healing process. Such wound models have been employed for quantitative and qualitative studies of wound healing, such as granuloma breaking strength and hydroxyproline content. Gain in granuloma breaking strength indicates increased collagen maturation by increased cross-linking; hydroxyproline estimation gives the net rate of synthesis and deposition of collagen in healing[11]. Significant increase in dry weight of granulation tissue (60.4±0.54; 52.5±0.65), tissue breaking strength (396±2.3; 357±2.2), and hydroxyproline content (2268±0.68; 1981±0.64) was recorded in the animals treated with methanol leaf extract followed by aqueous extract as depicted in Table 3. Histological studies of granulation tissue of the control group showed aggregation of more number of macrophages and less collagen fibres (fig. 1), whereas aqueous extract treated group animals revealed lesser macrophages and moderate collagenation (fig. 2). In methanol leaf extract treated animals, significant increase in collagen deposition with lesser macrophages (fig. 3) was noticed.

Wound healing comprises different phases such as contraction, epithelialization, granulation, and collagenation. Collagen is a major protein of the extracellular matrix and is the component that ultimately contributes to wound strength. Breakdown of collagen liberates free hydroxyproline. Measurement of the hydroxyproline could be used as an index for collagen turnover[12]. In the present study, significant increase in the hydroxyproline content of the granulation tissue of the animals treated with methanol leaf extract was recorded followed by aqueous extract when compared to control group. Increase in breaking strength of granulation tissue indicated the enhanced collagen maturation by increased cross-linking. In addition, increase in dry granulation tissue weight indicated the presence of higher protein content[13]. Increase in the tissue breaking strength, granulation tissue dry weight, and increased epithelialization could be attributed to the increased hydroxyproline content in the wound tissue[14].

Many workers studied the wound healing properties of several plants such as *Merremia tridentata*[15], *Datura alba*[16], *Coronopus didymus*[17] and *Aloe vera*[18]; the wound healing potency of these medicinal plants may be attributed to the active constituents present in it. Flavonoid reduces lipid peroxidation by preventing or slowing the onset of cell necrosis and by improving vascularity[19]. Tannins[20] and triterpenoids[21] are known to promote the wound healing process, mainly due to their astringent and antimicrobial property. These active constituents promote the process of wound healing by increasing the viability of collagen fibrils, by increasing the strength of collagen fibres either by increasing the circulation or by preventing the cell damage or by promoting the DNA synthesis[22].

The present study revealed that the methanol leaf extract of *Leucas hirta* possesses better wound healing potency, followed by aqueous extract, which was evident by the increased rate of wound contraction; reduction in the period of epithelialization; increase in collagen deposition, breaking strength, and hydroxyproline in granulation tissue. The potency of the plant in healing the wounds may be attributed to the phytoconstituents like flavonoids, alkaloids, tannins, saponins, glycosides, steroids, and triterpenoids present in it, which may be either due to their individual or additive effect, hastening the process of wound healing. The present investigation offers scientific evidence to the folkloric accounts of the use of leaf extract of *Leucas hirta* in treating cuts and wounds.

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