

his help in analyzing the data.

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Wound Healing Activity of *Chandanadi Yamak* in Rats

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Panchagavya is a term used in Ayurveda to describe the five important bovine products, milk, curd, ghee, urine and dung. Several formulations based on *panchagavya* are reported in Ayurvedic texts. One such formulation, *Chandanadi Yamak* was tested in the present study for its topical wound healing activity. Studies were conducted in male Wistar rats. Two wound models, incision wounds for tensile strength and excision wounds for wound contraction were employed along with histopathological evaluation. The application of the test formulation alone promoted wound contraction and reduced the time for wound closure showing healing potential comparable to marketed framycetin sulphate cream (1% w/w). The histological studies reveal complete healing with the test formulation showing good keratinization, epithelialization, fibrosis and angiogenesis. The present study demonstrates the wound healing potential of the test formulation.

Wounds are visible results of individual cell death or damage, and can be classified by site, size, depth, cause (surgery/accident) or circulatory failure¹. Wound healing is a process, which is fundamentally a connective tissue response. Initial stage of this process involves an acute inflammatory phase followed by synthesis of collagen and other extracellular macromolecules, which are later remodeled to, from a scar². Several factors delay or reduce wound healing, including bacterial infections, necrotic tissue, interference with blood supply, lymphatic blockage and diabetes mellitus. Generally if the above conditions could be altered

by any agent, an increased healing rate could be achieved³.

Chandanadi Yamak is a *panchagavya*-based polyherbal formulation, claimed to promote wound healing in traditional practices. *Panchagavya* is a term used to refer the five important bovine products, milk, curd, ghee, urine and dung. Several formulations based on *panchagavya* are reported in Ayurvedic texts and few reports concerning the evaluation of their pharmacological activities are reported in literature⁴⁻⁶. The present communication deals with the evaluation of wound healing activity of *Chandanadi Yamak* in terms of wound contracting ability, wound closure time, regeneration of tissues at wound site, tensile strength of wound and histopathological characteristics. The ingredients of

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TABLE 1: EFFECT OF TOPICAL APPLICATION OF CHANDANADI YAMAK ON EXCISION WOUNDS.

Post wounding days	Wounding area (mm ²)		
	Group I	Group II	Group III
0	303±5.0 (0)	308±43.2* (0)	305±6.4* (0)
3	275±5.0 (9.1)	282±4.5 (8.5)	257±3.5 (13.1)
6	230±9.2 (23.9)	210±3.0 (31.8)	174±6.5 (41.5)
9	189±5.0 (37.6)	131±5.2 (57.3)	105±8.2 (64.6)
12	130±8.3 (56.9)	46.2±2.5 (85.0)	39±3.0 (86.8)
15	74±4.3 (75.5)	5.4±3.0 (98.2)	0.0 (100)
18	41±6.2 (86.4)	0.0 (100)	0.0 (100)

Values are mean±SD of 6 animals in each group. Numbers in parenthesis indicates percent wound contraction. All values are significant at $p < 0.05$ as compared to Group I and *indicates not significant.

Chandanadi Yamak are *Pterocarpus santalinus*, *Ficus bengalensis*, *Nelumbo nucifera*, *Cynodon dactylon*, *Rubia cordifolia*, *Woodfordia fruticosa*, *Glycyrrhiza glabra*, *Sesame oil*, cow's milk and cow's *ghee*. The plants were authenticated with the help of a qualified botanist.

Chandanadi Yamak is a polyherbal formulation tested in the present communication for its wound healing activity. Chandanadi Yamak was obtained as a gift sample from Go-Vigyan Anusandhan Kendra, Nagpur.

Male Wistar rats (150-250 g) were used in the study. Animals were housed under standard conditions of temperature ($23 \pm 1^\circ$), 12 h light/dark cycle and fed with standard pellet diet (Gold Muhor brand, Lipton India Ltd.) and water *ad libitum*. Animals were acclimatized to laboratory conditions before commencement of experiment. Wounds were inflicted under light ether anaesthesia. Animals were then housed individually in clean polypropylene cages. The experimental protocols were approved by the Institutional Animal Ethics Committee [CPCSEA/02 II/01]. Animals in Group I received no treatment and served as control. Animals of Group II received application of Chandanadi Yamak (0.5 g) and animals in Group III received application of framycetin sulphate cream (FSC) 1% w/w (0.5 g). Except the drugs under study, no local/systemic therapy was provided to animals bearing any of the wounds.

Excision wounds were inflicted by excising a circular piece (300 mm² in area) of full thickness skin from the dorsal interscapular region as described previously⁷. Wound contraction was monitored by measuring wound area, planimetrically, every three days till the wounds were com-

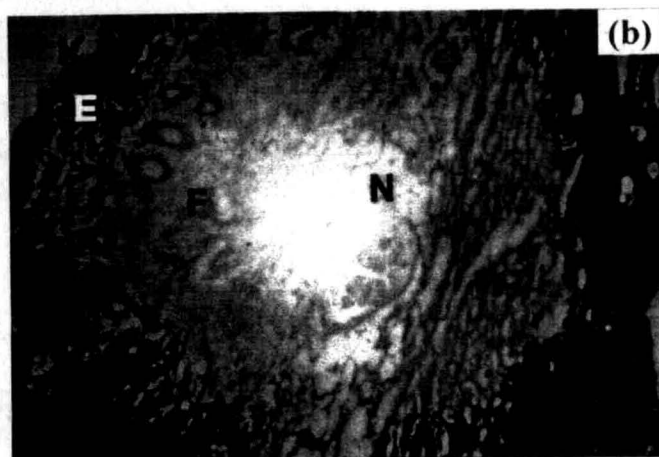
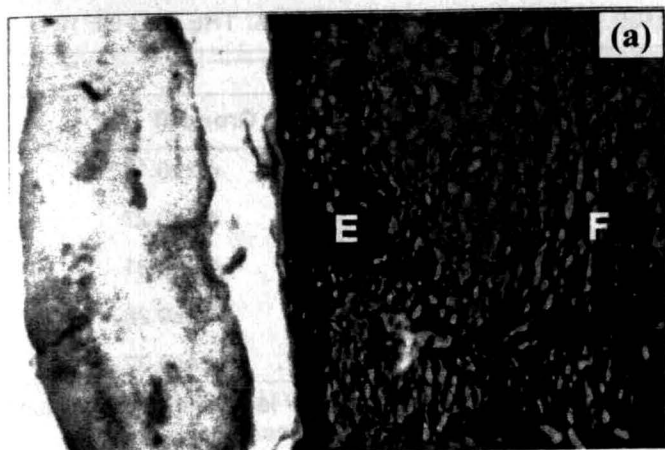
TABLE 2: EFFECT OF TOPICAL APPLICATION OF CHANDANADI YAMAK ON INCISION WOUNDS.

Group	Treatment	Tensile Strength (g)
I	Untreated	281±5.82
II	Chandanadi Yamak	295±4.21
III	FSC (1% w/w)	398±6.32*

Values are mean ±SD of 6 animals in each group. * $p < 0.05$ VS Group I.

pletely healed. Wound contraction was calculated as percent reduction in wound area. Incision wounds were made by two 5 cm long paravertebral incisions through the entire thickness of skin at a distance of about 1.5 cm from the midline on each side of depilated back of the rat⁸. After mopping the wound dry, intermittent sutures were placed 1 cm apart, using surgical nylon thread and a curved needle (No. 11). On day 7, sutures were removed and on day 10 the tensile strength was measured⁹.

Sections from the regenerated tissue (10 d) were observed under light microscope for keratinization, epithelialization, inflammation, fibrosis and neovascularization¹⁰. The results were visually quantified by numbering the results from 1 to 5, with 5 standing for maximum similarity and 1 standing for least similarity from the normal tissue around the wounded area in test and control wounds. Data was analyzed using one-way analysis of variance (ANOVA) followed by Toukai-Kramer multiple comparisons test. P values < 0.05 were considered significant.



The measurements of the progress of the wound healing of excision wound are recorded in Table 1. The percent closure of excision wound area was significantly increased by both *Chandanadi Yamak* and FSC as compared to control ($p < 0.05$). Treatment with *Chandanadi Yamak* showed comparable rate of wound contraction with the reference standard FSC. Although the wound contraction ceased at around day 15-18, the treatment was continued up to 24 d to monitor the fall of eschar, leaving no raw wound behind. The results suggest enhancement of wound contraction rate (between 15-18 d) and hastened epithelization as induced by fall of eschar.

Incision wound model showed increase in tensile strength of wounds due to treatment with FSC when compared to untreated control as shown in Table 2. The mean \pm S.D. tensile strength in control animals was 281.3 ± 5.82 whereas in *Chandanadi Yamak* and FSC treated rats tensile strength was 295.4 ± 4.21 and 398.0 ± 6.32 , respectively. The importance of cross-linking between collagen molecules and the physical weave of collagen fibres in contributing to the tensile strength of wounds is well acknowledged¹¹. Increase in tensile strength may be due to increase in collagen concentration per unit area and stabilization of fibres. Thus, treatment with *Chandanadi Yamak* does not seem to promote collagenation and therefore probably the tensile strength did not show any significant increase over the untreated group.

Histopathological studies show no evidence of keratinization and neovascularization in untreated control, although fibrous tissue proliferation and collagenation is evident. Good healing with prominent keratinization, epithelialization, neovascularization and fair fibrous tissue proliferation is shown with wounds treated with *Chandanadi Yamak* and

Fig. 1: Histology of regenerated tissue of 10-d old wounds.

(a) Untreated control from group I. (b) *Chandanadi Yamak* treated animals in group II and (c) framycetin sulphate cream 1% w/w treated group III. Magnification is X100. K stands for keratinization, E indicates epithelialization, F stands for fibrosis and N indicates neovascularization.

FSC (fig. 1). Keratinization, epithelialization and neovascularization was prominent in the treated wounds compared to control untreated wounds (Table 3).

The results of the present study demonstrates that topical application of *Chandanadi Yamak* enhanced healing in excision wounds in rats. This is supported by histopathological studies. The process of wound healing occurs in different phases such as coagulation, contraction, epithelialization, granulation, collagenation and tissue remodeling. The present study indicate the wound healing potential of *Chandanadi Yamak* by promoting the wound contraction and healing processes like epithelialization and fibrosis. The activ-

TABLE 3: HISTOLOGICAL EXAMINATION OF WOUNDS TREATED WITH *CHANDANADI YAMAK* AT THE END OF 10 D.

Parameter	Treatment		
	Group I	Group II	Group III
Keratinization	0.2±0.13	4.0±0.21	4.2±0.10
Epithelization	1.5±0.31	3.9±0.30	4.0±0.27
Fibrosis	2.2±0.32	3.7±0.12	4.0±0.31
Collagen	2.5±0.41	2.0±0.33	4.2±0.28
Neovascularization	0.5±0.25	4.3±0.10	4.4±0.37

Values are mean±SD from 6 readings each. Value 5 refers to maximum similarity and 0 refers for least similarity of wound from the normal tissue. All values are significant at $p < 0.05$.

ity is comparable to FSC, which exhibits potent antimicrobial action. However, *Chandanadi Yamak* did not promote the tensile strength as indicated by the results of incision wound model. Herbs like *Glycyrrhiza glabra* and *Nelumbo nucifera*, present in *Chandanadi Yamak*, exhibit potent antimicrobial activity^{12,13}. A more detailed study with regard to the antimicrobial potential of *Chandanadi Yamak* would give a deeper insight into its mechanism of action to consider its clinical use.

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