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STUDIES ON WOUND HEALING ACTIVITY OF GEL FORMULATION CONTAINING COW GHEE AND ALOE VERA

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ABSTRACT

The present study aims to evaluate the wound healing activity of gel containing cow ghee and aloe vera in rats. Incision wounds for tensile strength and excision wounds contraction along with the histopathological examination of the regenerated tissues were employed to investigate the wound healing potential. Topical application of the test formulation alone promoted the tensile strength (incision wounds) and wound contraction (excision wounds) showing healing potential comparable to framycetin sulphate cream (1%w/w). Histological examination reveals good keratinization, epithelization, fibrosis and collagenation indicative of the wound healing potential of gel. The present study thus offers a valuable insight into the claimed wound healing potential of the test formulation.

INTRODUCTION: Wound may be defined as a loss or breaking of cellular and anatomic or functional continuity of living tissue[1]. Wound healing is a process which is fundamentally a connective tissue response, initial stage of this process involves an acute inflammatory phase followed by the synthesis of collagen and other extracellular macromolecules which are later remodeled to form scar [2]. Wound healing involves a highly dynamic integrated series of cellular, physiological and biochemical process which occur in living organism. Several factors delay or reduce wound healing including bacterial infection, necrotic tissue, and interference with blood supply, lymphatic blockage and diabetes mellitus. Generally if the above factors could be altered by any agent, an increased healing rate could be achieved[3]. This is ayurvedic herbal formulation investigated for their pharmacological actions and clinical uses [4, 5].

MATERIALS AND METHODS:

GEL FORMULATION:

Design of basic formulation: It is reported that inclusion of cow's ghee in topical formulation helps the process of wound healing. But the claims mentioned for the use of cow's ghee for wound healing is yet to be found out. Therefore, the gel formulation containing 1%, 5%, 10% and 20% of old ghee were prepared during the preliminary stages in order to determine the maximum concentration of ghee that can be easily incorporated. But during stability studies it was observed that at a temperature above 40^0c ghee oozes out from the gel containing 10% of ghee, though it was stable at room temperature. Therefore it was decided to incorporate 5% of ghee in gel formulations for further studies.

METHOD: Gel formulation was prepared in following manner:

1% carbapol -934F was dissolved in small quantity of water and kept for hydration over night. Next day a resultant mixture was vigorously mixed using mechanical stirrer. Aloe vera and other water-soluble ingredients except triethonolamine and ghee, mentioned in formula were subsequently added with continuous stirring. When a homogenous mixture was formed, triethanolamine was added dropwise with gentle stirring, in the mixtures, to avoid any entrapment of air, which is often a problem once gelling occurs. At the end 5% of melted ghee was added into formulation with little stirring till the homogenous mixture was formed, which was then transformed into amber colored bottles and stored in subdued light.

Formula of gel formulation:

Plain ghee-5%, Aloe vera gel-0.25%, Propylene glycol, Glycerin, Disodium EDTA, Sodium metabisulphate, Propyl paraben, Carbapol-934F, Triethanolamine, Water up to 100%.

Animals: Male wistar rats (150-200g) were used in the study. Animals were housed under standard conditions of temperature (23 ± 1^0), 12h light/dark cycle and fed with standard pellet diet (gold mohar brand, lipton india ltd.) and water ad libitum.

Treatment: Animals were acclimatized to laboratory conditions before

compensement 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 institute animal's ethics committee. Animals in group I received no treatment and served as control. Animals of group II received application of gel formulation (0.5g) and animals in group III received application of framycetin sulphate cream (FSC) 1% W/W (0.5g).

WOUND MODELS:

Excision wounds: A circular piece (300mm^2 in area) of full thickness skin was excised from the dorsal interscapular region. Wound contraction was monitored by measuring wound area, on alternate days til the wounds were completely healed.[6] Wound contraction was calculated as percentage reduction in wound area. The wound half closure time WC-50, was calculated by litchified and wileoxon method. The time taken for epithelization was measured in days indicated by fall of eschar leaving no raw wound behind[7].

Incision wound: Incision wounds were inflicted by the method of Ehrlich and hunt Two 5 cm long paravertebral incision were made through the entire thickness of skin at a distance of about 1.5 cm from midline on each side of depilated back sutures of the rat. After mopping the wound dry, intermittent sutures were placed 1cm apart, using surgical nylon thread and a curved needle (No.11) and on 10th day tensile strength was measured[8].

METHODS:

Tensile strength measurement: Six groups containing six rats in each were used. Incision wounds were inflected in rats under light ether anesthesia. A 4 cm. long incision was made through the entire thickness of the skin in each rat on its depilated back. The wounds were mopped dry. The wounds were closed with intermittent sutures of surgical nylon thread and curved needle no.11, 0.5cm apart, which was removed on the 7th post wounding day. On 10th post wounding day, the tensile strength of skin was measured by method Lee.

Histopathological studies: Six groups containing six rats in each were used. Incision wounds were inflected in rats under light ether anesthesia. A 4 cm inch incision was made through the entire thickness of skin in each rat on its depilated back. The wounds were mopped dry. The wounds were closed with intermittent sutures by surgical nylon thread and curved needle no. 11, 0.5 cm apart, which were removed on the 7th post wounding day, small pieces of skin were excised from the rats under light ether anesthesia in such a way that each pieces represented the skin surroundings the incision originally made. The sections of the skin were stained with eosin and hematoxylin and were examined microscopically for keratinization, epithelization, fibrosis and angiogenesis.

Wound contraction studies: Six groups containing six rats in each were used. The skin of the impressed area on the depilated back of each rat was excised to the full thickness under light ether anesthesia to obtain a circular wound

area about 300mm^2. Wound contraction was monitored by measuring wound area which was traced on transparent polythene paper. Later the wound area was assessed using a graph paper. Wound contraction was also expressed as the percentage decrease of original wound size on every alternate day [9, 10].

Table 1: Effect of topical application of gel formulation on excision wounds

Post wounding days	Group I	Wounding area (mm^2) Group II	Group III
0	378.46 ± 9.26 (0)	355.98±6.96 (0)	345.05±6.63 (0)
3	280.28± 8.21 (25.94)	284.20± 9.90 (10. 39)	300.05 ± 6.63 (12.94)
6	245.66 ±8.21 (35.08)	259.00 ±3.49 (15.35)	243.43±5.41 (29.45
9	209.93 ± 7.79 (44.53)	204.30 ± 0.86 (33.23)	199.88 ± 2.93 (42.07)
12	193.18 ± 6.31 (48.95)	187.6 ± 7.23 (38.68)	161.91 ±5.06
15	176.66 ± 3.05 (53.40)	132.80 ± 8.21 (56.59)	120.60 ± 4.47 (65.04)
18	151.10 ± 1.54 (60.07)	77.43 ± 0.38 (74.69)	80.2 ± 0.44 (76.75)
21	121.56 ± 0.41 (67.88)	28.60 ± 0.43 (90.65)	29.80 ±0.08 (91.36)
24	101.23 ± 0.15 (73.25)	8.8 ±0.08 (97.12)	0.0 (100)
27	60.7 ±0.08 (83.96)	0.0 (100)	0.0 (100)

Table 2: Histopathological examination of wounds treated with gel at end of 10 Days

Parameter	Group I	Group II	Group III
Keratinization	0.2±0.13	1.0±0.12	4.2±0.18
Epithelization	1.5±0.31	4.2±0.20	4.0±0.27
Fibrosis	2.2±0.32	4.3±0.25	4.0±0.37
Collagen	2.5±0.41	3.8±0.18	4.2±0.28
Neovascularization	0.5±0.25	3.2±0.16	4.4±0.37

STATISTICAL ANALYSIS: Data was analysed using one way analysis variance (ANOVA) followed by tukey-kramer multiple comparison test. P values < 0.05 were considered significant.

RESULT AND DISCUSSION: The results of the progress of wound healing on excision wound are recorded in table I. On treatment with gel, the results are comparable with that of FSC 1%w/w/ showing better healing compared to control (P<0.05). The wound contraction ceased at around day 21-24 and treatment was continued up to day 30 to monitor the fall of eschar leaving no raw wound behind. The results obtained indicate enhancement of wound contraction rate between day 21-24 and increased epithelization followed by fall of escha. With the incision wound model, results of tensile strength are shown in table II. It is seen that the wounds treated with gel have the nearly same tensile strength with that of FSC showing significant increase over untreated control.

The mean ± SD of tensile strength in control animals was 281.30±5.82 whereas in gel it is 316.66±6.36 and in FSC 398.0±6.32. A significant reduction in the period of epithelization was observed when compared with group I. significant increase in tensile strength that the gel promotes collagen formation. The importance of croos linking between collagen molecules and physical means of collagen fibres in contributing to the tensile strength of wounds is well acknowledged.

Histopathological observations of the regenerated tissues revealed

incomplete healing with poor keratinization, epithelization, and fibrosis and collagen formation in untreated rats. Marked improvement in the healing parameters was observed due to treatment with gel as indicated by the results shown in table II. Although keratinisation was not highly pronounced, other healing parameters like collagenation, fibrosis and epithelization were promoted due to treatment with gel. The results are comparable to FSC 1%w/w. Wound healing involves different phases such as contraction, epithelization, granulation, collagenation and tissue remodeling. The present study indicates wound healing potential of gel by promoting the wound contraction and healing processes. The activity is comparable with FSC 1%w/w which predominantly shows antimicrobial action.

From the above results we can conclude that the gel promotes healing of incision and excision wounds rationalizing its traditional claim.

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