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DEVELOPMENT AND EVALUATION OF HERBAL WOUND HEALING FORMULATIONS

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ABSTRACT: The aerial parts of *Centella asiatica* (L.) Urban and rhizomes *Curcuma longa* (L.) were collected, dried under shade and extracted with ethanol and water respectively. These extracts were dried. *Aloe barbadensis* concentrated gel powder was obtained as free sample. Each extract and Aloe gel was added with appropriate proportion in cream base and evaluated for wound healing activity against povidone iodine ointment. In excision wound model the complete wound healing was observed with cream formulation I and II treated rats was observed in 18 days where as povidone iodine ointment took 16 days. In incision wound model tensile strength of formulations I and II was found to be 366.08±2.32 and 351.35±3.29 gm respectively. Tensile strength of povidone iodine ointment was found 379.98±2.95 gm. Herbal formulation I and II took two days more for complete wound healing as compared to povidone iodine ointment.
KEYWORDS *Centella asiatica*, Cream formulation, wound healing activity.

INTRODUCTION

Wound is the disruption of cellular and anatomic and functional continuity of living tissue, produced by physical, chemical, electrical or microbial insults to the tissue[1]. Wound healing is the dynamic process take place by regeneration or repair of broken tissue[2].

Normal wound-healing response begins with injury and is a concerted sequence of events. The healing cascade is activated when platelets come into contact with exposed collagen leading to platelet aggregation and the release of clotting factors resulting in the deposition of a fibrin clot at the site of injury. The fibrin clot serves as a provisional matrix and sets the stage for the subsequent events of healing[3]. Inflammatory cells also arrive along with the platelets at the site of injury providing key signals known as cytokines or growth factors[4]. The fibroblast is the connective tissue responsible for collagen deposition that is needed to repair the tissue injury[5]. In normal tissues, collagen provides strength, integrity, and structure. When tissues are disrupted following injury, collagen is needed to repair the defect and restore anatomic structure and function[6]. In the present work formulations were prepared to accelerate wound healing process.

Literature survey revealed that *Centella asiatica* has the antimicrobial[7], antioxidant activity[8], wound healing property[9] and increase collagen synthesis and tensile strength[10]. *Curcuma longa* has anti-inflammatory, antimicrobial[11] and wound healing property[12]. *Aloe* has the anti- inflammatory activity[13], increase degree of cross-linking[14]. Aloe also provides moisturizing activity.

In present study for easy application on wound o/w cream were prepared and their feasibility was checked by wound healing activity in wistar rats as compared to povidone iodine ointment.

MATERIAL AND METHOD

Preparation of extract

Centella asiatica (L.) Urban and *Curcuma longa* (L.) were collected from Amravati. The Plant species were identified and confirmed by Botanist Dr.Prabha Bhogaonkar, Director, Govt. Vidarbha institute of science and humanities, Amravati. A herbarium was prepared and deposited at Dept. of Botany, Govt. Vidarbha institute of science and humanities, Amravati.

Air-dried aerial parts of *Centella asiatica* (L.) Urban were defatted with petroleum ether and extracted with 90% ethanol in Soxhelet apparatus for 8 hours. The extract was concentrated on water bath and dried at 50^0 $\pm5^0$C in hot air oven. The powder of *Curcuma longa* rhizomes was boiled in purified water for 2 hours. The solution was then filtered through a coarse sieve twice.

Finally, the filtrate was concentrated on water bath to a thick paste like consistency and dried at $50^0 \pm 5^0$C in hot air oven.

Aloe barbadensis concentrate gel powder was obtained as a free sample from Chaitannya Biologicals Private Limited, Malkapur (M.S.).

Preparation of Formulation

Table No. 1 Formulation I and II

Ingredients	**Formulation I quantity (g)**	**Formulation II quantity (g)**
Centella asiatica extract	6	5
Curcuma longa extract	1	0.5
Aloe barbadensis Gel	2	2
Polysorbate 60	5	5
White Soft Paraffin	25	25
Cetosteryl alcohol	4	4
Glycerin	12	12
Methyl paraben	0.1	0.1
Propyl paraben	0.05	0.05
Butylated hydroxyl anisole	0.02	0.02
Purified water	q.s.to 100 gm	q.s.to 100 gm

Procedure

Aloe barbadensis concentrate gel powder was added in purified water and kept overnight. Phase I was prepared by dissolving *Centella asiatica* extract, *Curcuma longa* extract and above prepared aloe gel in purified water and previously heat to then glycerin was mixed the solution with constant stirring.

Phase II was prepared by melting white soft paraffin at 70^0C, then cetosteryl alcohol and polysorbate 60 was added to it with constant stirring. Lastly Methyl and propyl paraben and Butylated hydroxyl anisole (BHA) were added the mixture was stirred.

Phase II was added to Phase I with constant stirring at 70^0C. Then allow cooling with constant stirring.

EVALUATION OF WOUND HEALING ACTIVITY

The experimental protocol was approved by institutional animal ethical committee of Anuradha College of Pharmacy, Chikhli Dist. Buldana as per guidelines issued by Committee of Purpose of Control and Supervision of Experiment on Animals (CPCSEA) India.

Animals

Male wistar rats (150-200g) were used for study. All animals had free access to pelleted food and water *ad libitum*. Temperature was maintained at 23± 1^oC.

Treatment

Animals were wounded under light ether anesthesia, semiaseptically. The animals were assigned into five group (n=6). Group I was untreated group, this was taken as control. Group II animals were received Povidone iodine ointment treatment. Group III animals was received Cream base treatment, while groups of IV and V animals were received test formulations I and II treatments respectively in both excision and incision wound models. No other topical or systemic therapy was given to animals during the course of this study.

1) Excision wound model[15]

Hairs were removed from dorsal thoracic central region of anaesthetized rats. Full thickness from the demarketed area was excised to produce wound measuring around 300 mm^2. Wound was cleaned with cotton swab soaked in alcohol. The two test formulations, cream base and povidone iodine ointment were applied on wound once daily for 20 days starting from the first day of wounding. Wound contraction was measured for 20 days at interval of 2 days.

2) Incision wound model[16]

Animals were anaesthetized and paravertebral incisions (2.5-3.0 cm long) were made through the entire length of skin. After the incision was made, the parted skin was kept together and stitched with nylon thread at 0.5 cm apart with curved needle (No. 11). The two test formulations, cream base and povidone iodine ointment

were applied on wound once daily for 7 day. The sutures were removed on day 8 and wound tensile strength was measured on day 10 by using constant water flow technique.

Constant water flow technique

On the 10th day the animals was secured to the operation table, under light ether anaesthesia. A line was drawn on normal skin on either side of wound, 3 mm away from the wound line. Two Allis forceps were firmly applied on the lines facing each other. On one side the forceps was hooked firmly to metal rod fixed to the operation table. The other forceps was connected to a leakproff graduated polythene container through a string running over a pulley. The polythene container was connected to water reservoir placed at suitable height through a rubber tube kept occluded with a pinchcock. To measure wound tensile strength, the tube was released to allow a constant and continuous flow of water from the reservoir in to the polythene container. As the weight gradually increases, it acts as a pulling force to disrupt the wound. As soon as the gapping of the wound was observed, the rubber tube was clamped and the polythene container was weighed.

Table No. 2 Excision wound model

Post woun -ding days	Wound Area(mm^2)				
	Control (Group I)	**Standard (Group I)**	**Cream base (Group I)**	**Formulation I (Group I)**	**Formulation II (Group I)**
0	330.58 ±18.20 (0)	325.11 ±10.51 (0)	334.86 ±18.13 (0)	324.06 ±13.25 (0)	327.80 ±17.66 (0)
2	270.83 ±4.30 (18.07)	229.58 ±3.35 (29.38)	266.98 ±2.22 (20.27)	234.65 ±6.20 (27.59)	239.90 ±5.46 (26.81)
4	248.03 ±4.72 (25.03)	195.88 ±4.83 (39.74)	236.31 ±5.23 (29.43)	271.26 ±2.13 (32.95)	221.90 ±2.01 (32.30)
6	232.03 ±1.72 (30.17)	178.38 ±1.89 (45.13)	225.48 ±5.06 (32.66)	182.65 ±2.25 (43.63)	183.28 ±2.45 (44.39)
8	194.71 ±2.59 (41.10)	129.73 ±2.32 (60.09)	187.61 ±1.50 (43.97)	135.21 ±2.94 (58.27)	145.85 ±2.41 (55.50)
10	184.60 ±2.53 (44.15)	100.58 ±2.36 (69.06)	174.95 ±3.20 (47.75)	114.85 ±2.07 (64.55)	122.00 ±1.63 (62.78)
12	169.25 ±2.15 (48.80)	63.88 ±1.64 (80.35)	149.83 ±1.56 (55.25)	79.58 ±1.65 (75.44)	87.53 ±3.41 (73.29)
14	152.96 ±2.12 (53.72)	21.5 ±1.59 (93.38)	124.01 ±3.17 (62.96)	36.73 ±2.60 (88.66)	58.11 ±1.75 (82.27)
16	122.30 ±3.44 (63.00)	**0(100)**	107.10 ±2.45 (68.01)	17.08 ±2.36 (94.72)	26.45 ±1.66 (91.93)
18	108.65 ±2.70 (67.13)	0(100)	74.06 ±2.12 (77.88)	**0(100)**	**0(100)**
20	93.53 ±1.57 (71.68)	0(100)	56.46 ±1.79 (83.13)	0(100)	0(100)

Value are mean±SEM of Six animals (n=6) in each group. Number in parenthesis indicates percentage of wound contraction. All are significant at $p<0.05$ as compared to group I (control) and indicate not significant.

Table No. 3 (Incision wound model)

Group	Treatment	Tensile strength(gm)
1	Control (Group I)	164.55±3.14
2	Povidone iodine ointment (Group II)	379.98±2.95
3	Cream Base (Group III)	202.75±3.04
4	Formulation I (Group IV)	366.08±2.32
5	Formulation II (Group V)	351.35±3.29

Values are mean ± SEM of six animals in each group $p<0.05$ Vs group I.

RESULTS

In excision wound study (see table no.2), wound contraction progresses identically with Povidone iodine ointment and in wound treated with formulation I and II. In these three groups complete healing was observed between 16th and 18th day. While untreated group I (control) and group III (cream base) animals took more than 20 days for healing of wounds.

In incision wound study (see table no. 3), tensile strength of Povidone iodine ointment was found 379.98±2.95 gm and group I (control) was found 164.55±3.14 gm, while the group III (cream base) showed 202.75±3.04 gm. Tensile strength of formulations I and II showed 366.08±2.32 and 351.35±3.29 gm respectively.

Statistical analysis

The results were expressed as mean± SEM. The significance of differences between the means was analyzed by student's t- test followed by Turkey's test. A P-value<0.05 was considered significant.

DISCUSSION

In the present work, plants like *C. asiatica, C. longa* and *A. barbadensis* were selected which accelerate wound healing process. The formulation I and II showed best results this may be due to asiaticoside from *C. asiatica* accelerate collagenisation and tensile strength[10]. Asiaticoside, a major constituent of the herb is know to promoted wound-healing by reducing lipid peroxide levels in wounds[9]. Curcumin from *C. longa* is responsible for closure of wound and increase in collagen synthesis[12]. *A. barbadensis* increased degree of cross-linking as seen by increased aldehyde content and decreased acid solubility[14]. These all factors may be responsible for wound healing activity of formulations I and II.

In incision wound study, formulations enhanced the tensile strength of 10 days old wounds as compared with wound of untreated group. The importance of cross linking between collagen molecules and physical weave of collagen fibers in contributing to the tensile strength of wound is well acknowledged. Increase in tensile strength may be due to increase in collagen concentration per unit area and stabilization of fibers. Formulations I and II showed significant increase in tensile strength of the 10 day old wound. This may be due to promotion of collagenation and cross linking.

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