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COMPARATIVE EFFICACY OF TWO POLYHERBAL CREAMS WITH FRAMYCETIN CREAM IN TREATING FRESH WOUNDS.

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ABSTRACT

Restoration of damaged tissue, wound or facture is an important process which plays vital role in survival of life. The medicinal plants used in formulation of Cream A (Ari's Wound Healing Cream) and Cream B (Amarantha Wound Healing Cream) have long been used in traditional Indian System of Medicine and serve as the principal herbs in treating wound. Market survey revealed that very few formulations are available in the market to enhance wound healing process. This prompted us to assess wound healing efficacy of two Polyherbal topical creams in albino rats. Wound healing activity was evaluated using incision and excision wound models in albino rats. The results demonstrated that on 16th day, the % wound contraction of excision wounds treated with cream A and cream B **(**91.86 $\pm$ 2.57 % and 98.13 $\pm$ 0.72 % respectively) was better than wound treated with Framycetin Sulphate cream (89.43 ±3.280%). Epithelialization period of wounds treated with cream A (day 23) and cream B (day 17) was found to be significantly ($p<0.05$, $p<0.001$ respectively) lower as compared to wounds treated with Framycetin Sulphate cream (day 27). Breaking strength of wounds of animals treated with Cream A (583.33 ± 9.54) and Cream B (650 ± 18.25) was found to be lower as compared to tensile strength of incised wounds treated with standard (Framycetin sulphate

cream). Results of *in vivo* experiments indicate that polyherbal cream A and cream B exert significant wound healing activity, demonstrating its relevant therapeutic potential.

KEYWORDS: Polyherbal cream, Excision wound, Incision wound, Framycetin sulphate cream.

INTRODUCTION

A wound is defined as loss or breaking of cellular, anatomical or functional continuity of living tissues.[1] Wounds are inescapable events in life. Wound may be produced by physical, chemical, thermal, microbial or immunological insult to the tissue. Proper healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin.[2] Healing of wounds involves the activity of an intricate net work of blood cells, cytokines and growth factors which ultimately leads to the restoration to normal condition of the injured skin or tissue.[3] The four phases of normal wound healing include haemostasis, inflammation, proliferation and remodeling. Alteration in any of these steps can lead to delay or inability in dermal wound healing.[4]

Though healing process takes place by itself and does not require much help, various physiologic and mechanical factors such as poor nutrition, insufficient oxygenation, infection, prolonged inflammation, age, diabetes and oxidative stress[5] may impair the healing response and healing is delayed. In the clinical setting, delay in healing results in a chronic wound that fails to proceed through the usual stepwise progression and further may results in severe complications such as gangrene, which may leads to extended hospitalization, amputation and even death of patient.[6] Hence wound healing forms an important part of medical care of wound management and should be accelerated and controlled.

Nature has gifted us with many herbs having mystical healing properties that are used widely in number of ailments. The use of herbs and medicinal plants as the first medicine is a universal phenomenon. Today, as much as 80% of the world's population depends on traditional medicine as primary health care needs.[5] Plants and their extracts have immense potential for the management and treatment of wounds. A large number of plants/plant extracts/decoctions or pastes are equally used by tribals and folklore traditions in India for treatment of cuts, wounds, and burns.[7] The phytomedicines for wound healing are safe, effective, with less side effects, rare hypersensitive reactions and cost effective.[2] These

natural agents induce healing and regeneration of the lost tissue by multiple mechanisms such as disinfections, debridement, antioxidant and provide a moist environment to establish suitable environment for natural healing process.[5]

Literature survey revealed that many plants are known to have antioxidant, antimicrobial, anti-inflammatory and wound healing activities. Eg. *Aloe vera*, Turmeric, Neem.[8] We searched for such medicinal plants from Ayurvedic literature and practiced following the reverse pharmacology path. This led us to a shortlist of plant materials from almost 200 different options.

The present study was carried out to evaluate comparative efficacy viz. cream A (Ari's Wound Healing Cream) and cream B (Amarantha Wound Healing Cream) with Framycetin sulphate cream in fresh wounds. The cream A was prepared by using extracts of 8 herbs viz. *Glycyrrhiza glabra, Ficus infectoria, Shorea robusta, Curcuma longa, Berberis aristata, Rubia cordifolia, Azadirachta indica,Pongamia glabra* and Yashad Bhasma as Classical Ayurvedic Preperation. The cream B was prepared by using Jatyadi oil, Yashad Bhasma and extracts of seven herbs viz. *Ficus religiosa, Ficus bengalensis, Centella asiatica, Shorea robusta, Glycyrrhiza glabra, Azadirachta indica* and *Pongamia glabra*. The cream A differs from cream B in the form of quantity and types of ingredients used in the formulation. The major differentiator factors between two creams are presence of Jatyadi Oil and Mandukparni extract in Cream B. Also the percentage of Yashad Bhasma in cream B is more than cream A. Both the creams have Vranaropak properties.

The selected ingredients of both formulations were reported to have significant antimicrobial, antioxidant, wound healing and anti-inflammatory activities. The literature survey scientifically revealed the use of Jatyadi Taila in the management of wounds.[9] Yashad Bhasma is a Classical Ayurvedic Formulation which plays a significant role in protein synthesis, in cell division and in wound healing. It is known to have antiseptic and astringent properties.[10] The plant ingredients such as *Curcuma longa*, *Berberis aristata*, *Azadirachta indica*, *Pongamia glabra*and *Shorea robusta* possess antimicrobial and wound healing properties.[11] The plant ingredients such as *Ficus religiosa* and *Ficus bengalensis* help to constrict and heal the wounds due to their astrigent property.[12-13] Few herbs such as *Centella asiatica* have ability to heal the wounds by increasing synthesis of collagen and intracellular firbonectin content.[14] *Glycyrrhiza glabra* has also been used in the treatment of wounds, ulcers and burns.[15]

Market survey revealed that very few formulations are available in market to enhance wound healing process. Hence there is dire need to prepare safe, cost effective and plant based formulation for wound healing activity. This prompted us to assess wound healing efficacy of Cream A and Cream B by using incision and excision wound models.

MATERIALS AND METHODS

Materials

Cream A and Cream B were developed and supplied by Ari Healthcare Pvt. Ltd., Pune.

Composition of cream A (Ari's Wound Healing Cream)

Sr. No.	Ingredients	Botanical Name	Quantity
1	Yashtimadhuka Extract	*Glycyrrhiza glabra*	4 %
2	Plaksha Extract	*Ficus infectoria*	3 %
3	Shala Extract	*Shorea robusta*	3 %
4	Haridra Extract	*Curcuma longa*	2 %
5	Daruharidra Extract	*Berberis aristata*	2 %
6	Manjishtha Extract	*Rubia cordifolia*	2 %
7	Nimba Extract	*Azadirachta indica*	2 %
8	Karanja Extract	*Pongamia glabra*	1 %
9	Yashad Bhasma	Ayurvedic Classical Formulation	0.3 %
Cream base: QS to make 100%			

Composition of cream B (Amarantha Wound Healing Cream)

Sr. No.	Ingredients	Botanical Name	Quantity
1	Jatyadi Taila	Ayurvedic Classical Formulation	4 %
2	Ashvattha Extract	*Ficus religiosa*	3 %
3	Nyagrodha Extract	*Ficus bengalensis*	2 %
4	Mandukaparni Extract	*Centella asiatica*	3 %
5	Shala Extract	*Shorea robusta*	3 %
6	Yashtimadhuka Extract	*Glycyrrhiza glabra*	2 %
7	Nimba Extract	*Azadirachta indica*	1 %
8	Karanja Extract	*Pongamia glabra*	1 %
9	Yashad Bhasma	Ayurvedic Classical Formulation	1.5 %
Cream base: QS to make 100%			

Experimental animals

Albino Wistar rats of either sex weighing 180–200g were used for the study. The animals were procured from Haffkine Biopharmaceuticals, Mumbai. All animals were housed in polypropylene cages under standard experimental conditions with 26 ± 2^{0}C ambient temperature and 12 h light-dark cycle. The animals were fed standard pellet diet and were provided water *ad libitum*. All experimental protocols were approved by the Institutional

Animal Ethics Committee (CUSCP/IAEC/28/2011-12) of C. U. Shah College of Pharmacy, Santacruz (W).

Safety evaluation (Skin irritation study)

In order to evaluate safety of cream A and cream B, Skin irritation study was conducted on albino rats as per OECD guidelines No. 404 (OECD, 2004).[16] The back of the albino rats was shaved to remove the fur carefully, 24 hours before application of the sample. Cream A and B were applied on the skin patches of albino rats and the site of application in terms of erythema and edema was examined at 24, 48 and 72 hours for changes in any dermal reactions. The irritation index was calculated to assess the irritation potential of the cream A and B according to Draize Test.[17]

***In vivo* evaluation of wound healing**

Incision and excision wound models were used to evaluate the wound-healing activity of two polyherbal creams such as cream A and cream B in comparison with framycetin sulphate cream.

Grouping of animals

Animals were randomized into three groups of six animals each.

Group I : Received topical application of cream A,

Group II : Received topical application of cream B,

Group II : Received topical application of standard drug i.e. Framycetin sulphate cream (1 % w/w)

For both excision and incision wound models, the animal groups were classified and treated in the same manner.

Incision wound model

Animals were anaesthetized before wound creation. The particular skin area was shaved using hair remover cream (Veet) one day prior to the experiment. Incision of 6 cm was made through the skin and cutaneous muscles using sterile scissors and forceps. The incision was then closed with interrupted sutures with stitches 1cm apart using sterile absorbable sutures. Black surgical thread and curved needle no.19 were used for stitching wound. Cream A, Cream B and standard (Framycetin Sulphate cream) were topically applied once in a day.

The sutures were then removed on the 8th post – wounding day and the tensile strength of 10-day old wound was measured by tensiometer.[18]

Excision wound model

Animals were anaesthetized before wound creation. The particular skin area was shaved using hair remover cream (Veet) one day prior to the experiment. A full thickness of the excision wound of circular area (approx. 500 mm^2) and 2 mm depth was made on the shaved back of the rats. The wound was left undressed to the open environment. Cream A, Cream B and standard (Framycetin Sulphate cream) were topically applied once a day, starting from day 0 till complete epithelialization. The parameters studied were % wound closure and epithelialization time. Wound closure was measured as a percent contraction in wound area in each 4 days over a period of 30 days. Wound closure was studied by tracing the raw wound using transparent paper and a permanent marker on every 4th day for 16 days. Wound area was measured by retracing the wound on a millimeter scale graph paper. The period of epithelization was calculated as the number of days required for falling off of the dead tissue remnants without any residual raw wound.[19]

Percentage wound closure

$$\text{Percentage Wound Closure} = \frac{(\text{Initial area of Wound} - N^{TH} \text{ day area of wound})}{(\text{Initial area of Wound})} \times 100$$

Statistical analysis

Results were expressed as means ± SEM (Standard Error of The Mean). Comparisons between groups were performed using One way ANOVA followed by Bonferroni Multiple comparison test on GraphPad Instat 3 statistical software.

RESULTS AND DISCUSSION

The wound healing process consists of different phases such as granulation, collagenation, collagen maturation and scar maturation which are concurrent but independent of each other.[20] Therefore, it may not be possible to draw firm conclusion about the influence of a given agent on healing by studying only one phase of healing. Hence in the present study two different wound models were used to evaluate the wound healing efficacy of cream A and cream B in fresh wound models. The results of the present study showed that the Cream A and Cream B possessed a definite wound healing action.

Safety evaluation (Skin irritation study)

The results indicated that both the creams (Cream A and Cream B) did not cause any skin reaction after examining at 24, 48 and 72 hrs and can be classified as non-irritant. Both the creams were found to be safe for topical application. Cream A and Cream B showed no erythema or edema on intact rat skin. The primary skin irritation index of the creams was calculated as 0.00.

Incision Wound Model

The effect of wound healing activity was evaluated by determining the breaking strength of the incision wound of Cream A, Cream B and standard. The results were presented as mean weight in gram ± SEM required to open the sutured wound (Table 1). Breaking strength of wounds of the animals treated with Cream B was found to be greater as compared to tensile strength of wounds of the animals treated with Cream A. Thus, more weight and more strength is required to break the wounds treated with cream B as compared to wounds treated with cream A, indicating that cream B is more effective as compared to cream A. Breaking strength of wounds of the animals treated with standard (Framycetin Sulphate cream) was found to be significantly ($P<0.001$) greater than the tensile strength of wounds of the animals treated with cream A and cream B.

Table-1: Effect of topical application of Cream A, Cream B in comparison with Framycetin sulphate cream on breaking strength of the skin having incision wound.

Group	Breaking strength (g)
Cream A	583.33±9.545
Cream B	650.00±18.257
Standard	854.16±24.509***

Values are expressed in Mean ± SEM, n = 6

The treated groups are compared by Bonferroni Multiple comparison test with standard.

****P<0.001.*

Excision Wound Model

The effect of wound healing activity was evaluated by determining the **%** wound contraction and epithelialization period of the excision wound of Cream A, Cream B and standard. The results were presented as % wound contraction and epithelialization period (Mean± SEM) in Table 2. The studies on excision wound healing model revealed that both the groups (Cream A and Cream B) showed decreased wound area from day 0 to day 20. On 16th day, the % wound contraction of excision wounds treated with cream A and cream B **(**91.86±2.575 and

98.13±0.7242 %, respectively) was better than the wound treated with Framycetin Sulphate cream (89.43±3.280%). The healing of wounds was seen right from the post wound day 4 in animals treated with cream A and B.

The complete healing of wound was seen when the eschar falls. Wounds treated with cream B showed statistically significant (P<0.05, P<0.001 respectively) faster healing (day 17) as compared to wounds treated with cream A (day 23) and Framycetin Sulphate cream (day 27). This indicates that cream B promotes epithelialization of wound faster than cream A and Framycetin Sulphate cream.

Animals treated with Cream A and cream B showed enhanced rate of wound contraction and drastic reduction in healing time than Framycetin sulphate Cream, which might be due to enhance epithelialization and cellular proliferation. This enhanced epithelization may be due to the antioxidant effect of medicinal plants, which augments collagen synthesis. The period of epithelialization of animals treated with cream B and cream A was less than Framycetin Sulphate cream indicating faster wound healing. The animals treated with cream B showed significant results when compared with standard.

Table-2: Effect of topical application of Cream A, Cream B in comparison Framycetin sulphate cream on % wound Contraction and epithelialization period of excision wound models in rats.

Post Wounding Days	% wound Contraction		
	Cream A	Cream B	Framycetin Sulphate Cream
4	65.9±4.627	53.66±2.820	34.16±3.919
8	81.13±4.554	83.83±4.491	65.9±4.627
12	89.43±3.280	93.5±3.082	81.13±4.554
16	91.86±2.575	98.13±0.7242	89.43±3.280
20	96.1±1.055	100	91.86±2.575
24	100		96.1±1.255
Epithelialization period (days)	23±0.3651*	16.5±0.6191***	27±1.317

Values are expressed in Mean ± SEM, n = 6

The treated groups are compared by Bonferroni Multiple comparison test with standard.

**P<0.05, ***P<0.001*

The results of the present study showed that the cream B possessed significant wound healing activity than cream A is due to synergistic effects of Jatyadi Taila, *Centella asiatica* and other

ingredients present in the cream B. Asiaticoside isolated from *Centella asiatica* showed promising wound healing activity in normal as well as in diabetic animals.[21] Jatyadi Taila is a classical Ayurvedic formulation in oil form used for healing wounds. It is to be applied externally over non healing wounds, sinus, blisters, abscess, and bite wounds. It is also beneficial in burn wounds. It possesses antimicrobial activity due to that it acts as antiseptic and fungicidal. It is also useful in various skin afflictions. Wound healing efficacy of *Jatyadi Taila* was also evaluated scientifically by in vivo evaluation in rat using excision wound model.[9]

CONCLUSIONS

The study revealed the better activity of polyherbal creams (Cream A and Cream B) may be due to the synergistic action of the plants constituents present in the formulations. Thus, cream A and cream B possess a multifaceted approach in healing the wound.

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CONFLICTS OF INTEREST

Dr. Anisha S Kanjilal is Director of Ari Healthcare Pvt. Ltd. Mr. Sanjeevan S Kanjilal is Managing Director of Ari Healthcare Pvt. Ltd and Dr. Sanjay Nipanikar is Head - R & D Center of Ari Healthcare Pvt. Ltd., Pune. Ari Healthcare Pvt. Ltd. has developed and supplied Cream A and Cream B to C U Shah College of Pharmacy, Mumbai for research purpose. Dr. Pratima Takte and Minakshi N Nehete do not have any conflict of interest.

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