



Original research article (Experimental)

Comparative efficacy of two polyherbal creams with framycetin sulfate on diabetic wound model in rats



Minakshi N. Nehete^a, Sanjay Nipanikar^b, Anisha S. Kanjilal^b, Sanjivan Kanjilal^b,
Pratima A. Tatke^{a,*}

^a C. U. Shah College of Pharmacy, S.N.D.T. Women's University, Santacruz (W), Mumbai, India

^b Ari Healthcare Pvt. Ltd., Pune, Maharashtra, India

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ABSTRACT

Background: Diabetes mellitus is one of the metabolic disorders that impede normal steps of wound healing process. Worldwide, 15% of the 200 million diabetics suffer from diabetic wounds. Diabetic complications, such as foot ulcer, impose major public health burdens worldwide.

Objective: The present study was carried out to evaluate comparative efficacy of polyherbal creams with framycetin sulfate cream on diabetic rats using incision and excision wound models.

Materials and methods: Alloxan (120 mg/kg, intraperitoneal) induced diabetic rat models (incision and excision models) were used to evaluate wound healing effect of cream A, B, and framycetin sulfate. Cream A and B were applied for a period of 10 and 20 days for incision and excision wound models, respectively. Incision wound model was used to assess the effect on breaking strength. Wound contraction and epithelialization period were measured using excision wound model. The data were analyzed by one-way ANOVA followed by Bonferroni post-test.

Results: Tensile strength of the animals treated with cream B (941.66 ± 15.36) was found to be significantly greater ($P < 0.001$) as compared to tensile strength of the animals treated with cream A (825 ± 22.36). Wound treated with cream B was found to heal significantly ($P < 0.001$) faster (day 17) as compared to wounds treated with framycetin sulfate (day 21).

Conclusions: Cream B was found to be more effective wound healing agent than cream A and framycetin sulfate cream in treating diabetic wounds.

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1. Introduction

A wound is defined as loss or breaking of cellular, anatomical, or functional continuity of living tissues [1]. It is a clinical entity, is as old as human, and is often considered as major problem in clinical practice. Healing of wounds involve the activity of an intricate network of blood cells, cytokines, and growth factors which ultimately leads to the restoration of the injured skin or tissue to normal condition. The classic model of wound healing is divided into three sequential phases: Inflammatory phase (consisting of

hemostasis and inflammation), proliferative phase (consisting of granulation, contraction, and epithelialization), and remodeling phase, which organizes structure with increased tensile strength [2]. 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 other diseases, drugs, smoking, alcoholism, depression, and other factors [3] may impair the healing response, resulting in a chronic wound that fails to proceed through the usual stepwise progression.

Diabetes mellitus constitutes one of the most important public health problems due to its high prevalence and enormous social and economic consequences [4]. Delayed cutaneous wound healing is a chronic complication in diabetic patients and is caused primarily by hyperglycemia, prolonged inflammatory phase, defected angiogenesis, diminished expression of cytokines, oxidative stress, vascular insufficiency, and microbial infections [5–8]. Several

* Corresponding author. Pharmaceutical Chemistry, C. U. Shah College of Pharmacy, S.N.D.T. Women's University, Santacruz (W), Mumbai 400 049, Maharashtra, India.

E-mail address: patatke@gmail.com (P.A. Tatke).

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other diabetic complications such as neuropathy, nephropathy, atherosclerosis, and foot deformities contribute to the severity of the disease and in the development of chronic wounds in diabetic patients that might be complicated leading to ulceration, necrosis, and amputation [5,7]. Diabetes mellitus is life-threatening and becomes the third largest killer of humans after cancer and cardiovascular diseases, even with the use of several recent synthetic drugs for effective treatment options [9].

Healing impairment of diabetic patients is still a serious clinical problem for physicians worldwide due to unclear etiology. Hence, impaired wound healing in diabetics has caught the attention of the world to help promote healing process and prevent the rising complications. A new era in wound healing research is required, involving new treatment strategies to deal with this emerging issue. One of these is the use of medicinal plants as a source for natural products to explore new therapeutic agents/tools intended for diabetic wound management and treatment.

India has a rich tradition of plant-based knowledge on healthcare. Many Ayurvedic plants have a very important role in the process of wound healing. A large number of plants, plant extracts, decoctions, or pastes are equally used by tribals and folklore traditions in India for the treatment of cuts, wounds, and burns [10]. Plants are potent healers because they promote the repair mechanisms in a natural way [11]. The phytomedicines are safe, effective, and relatively cheap options for wound healing [12]. These natural agents induce healing and regeneration of the lost tissue by multiple mechanisms such as coagulation, disinfections, debridement, antioxidant, and provide suitable environment for natural healing process [3].

Literature survey revealed that many plants are known to have antioxidant, antimicrobial, anti-inflammatory, and wound healing properties for example, *Aloe vera*, turmeric, and neem [13]. We searched for such medicinal plants from Ayurvedic literature and followed the reverse pharmacology path. This led us to the short-listing 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 sulfate cream in diabetic wounds. Cream A was prepared using extracts of eight herbs viz., *Glycyrrhiza glabra*, *Ficus infectoria*, *Shorea robusta*, *Curcuma longa*, *Berberis aristata*, *Rubia cordifolia*, *Azadirachta indica*, *Pongamia glabra*, and Yashad Bhasma as classical Ayurvedic preparation. Cream B was prepared using Jatyadi Oil and classical Ayurvedic preparation, Yashad Bhasma and extracts of seven herbs viz., *Ficus religiosa*, *Ficus bengalensis*, *Centella asiatica*, *S. robusta*, *G. glabra*, *A. indica*, and *P. glabra*. Cream A differs from cream B in the form of quantity and types of ingredients used in the formulations. The major differentiating factors between the two creams are the presence of Jatyadi Oil and Mandukparni extract in cream B. Furthermore, 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 properties. The literature survey scientifically revealed the use of Jatyadi Oil in the management of wounds [14]. Yashad Bhasma is a classical Ayurvedic formulation which plays a significant role in protein synthesis, cell division, wound healing. It is known to have antiseptic and astringent properties [15]. The plant ingredients such as *C. longa*, *B. aristata*, *A. indica*, *P. Glabra*, and *S. robusta* possess antimicrobial and wound healing properties [16]. The plant ingredients such as *F. religiosa* and *F. bengalensis* help to constrict and heal the wounds due to their astringent property [17,18]. Few herbs such as *C. asiatica* have ability to heal the wounds by increasing synthesis of collagen and intracellular fibronectin content [19]. *G. glabra* has also been used in the treatment of wounds, ulcers, and burns [20].

The growing popularity of natural and herbal medications, easy availability of raw materials, cost-effectiveness, and paucity of reported adverse reaction, prompted us to assess wound healing efficacy of two Ayurvedic topical creams in comparison with framycetin sulfate cream.

2. Materials and methods

2.1. Materials

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

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

Ingredients	Botanical name	Part of plant	Types of extract	Quantity (%)
Yashtimadhuka extract	<i>Glycyrrhiza glabra</i>	Stem	Hydroalcohol	4
Plaksha extract	<i>Ficus infectoria</i>	Leaf and bark	Hydroalcohol	3
Shala extract	<i>Shorea robusta</i>	Bark	Hydroalcohol	3
Haridra extract	<i>Curcuma longa</i>	Rhizome	Alcohol	2
Daruharidra extract	<i>Berberis aristata</i>	Stem	Hydroalcohol	2
Manjishtha extract	<i>Rubia cordifolia</i>	Stem	Hydroalcohol	2
Nimba extract	<i>Azadirachta indica</i>	Leaf	Hydroalcohol	2
Karanja extract	<i>Pongamia glabra</i>	Bark	Hydroalcohol	1
Yashad Bhasma	Ayurvedic classical formulation	Powder	Powder formulation	0.3
Cream base: QS to make				100

Composition of cream B (Amarantha Wound Healing Cream): Each gram of cream contains percent of ingredients (w/w).

Ingredients	Botanical name	Part of plant	Types of extract	Quantity (%)
Jatyadi Oil	Ayurvedic classical formulation	Oil	Medicated oil	4
Ashvattha extract	<i>Ficus religiosa</i>	Stem	Hydroalcohol	3
Nyagrodha extract	<i>Ficus bengalensis</i>	Root	Hydroalcohol	2
Mandukaparni extract	<i>Centella asiatica</i>	Whole plant	Alcohol	3
Shala extract	<i>Shorea robusta</i>	Bark	Hydroalcohol	3
Yashtimadhuka extract	<i>Glycyrrhiza glabra</i>	Stem	Hydroalcohol	2
Nimba extract	<i>Azadirachta indica</i>	Leaf	Hydroalcohol	1
Karanja extract	<i>Pongamia glabra</i>	Bark	Hydroalcohol	1
Yashad Bhasma	Ayurvedic classical formulation	Powder	Powder formulation	1.5
Cream base: QS to make				100

2.2. Experimental animals

Albino Wistar rats of both sexes weighing 180–200 g 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 °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/27/2011-12) of C.U. Shah College of Pharmacy, Santacruz (W), Mumbai, India.

Table 1

Effect of topical application of cream A, B, and framycetin sulfate cream on tensile strength of the skin having incision wound in diabetic rat model.

Group	Tensile strength (g)±SEM
I	825 ± 22.36
II	941.66 ± 15.36***
III	920.83 ± 10.04**

Data were expressed as means ± SEM for six rats in each group. The treated groups were compared by one-way ANOVA with post-test. ** $P < 0.01$, *** $P < 0.001$ versus cream A. Group I: Cream A treated group, Group II: Cream B treated group, and Group III: Framycetin sulfate treated group, SEM: Standard error of mean.

2.3. Safety evaluation (skin irritation study)

To evaluate the safety of cream A and B, skin irritation study was conducted on albino rats as per OECD guide lines number 404 (OECD, 2004). Twenty four hours before application of the sample, the back of the albino rats was shaved carefully to remove the fur. 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 h for changes in any dermal reactions. The irritation index was calculated to assess the irritation potential of cream A and B according to Draize Test [21].

2.4. In vivo evaluation of wound healing activity

2.4.1. Induction of diabetes

Albino rats were weighed, and their fasting blood glucose levels were determined before inducing diabetes. The animals were then injected with single dose of alloxan (120 mg/kg, intraperitoneal). Fasting blood glucose levels were measured 3 days later to confirm the diabetic status of the animals. For blood glucose measurements, blood was withdrawn from the tail vein. Rats having blood sugar level above 250 mg/dl, were selected for study.

2.5. Grouping of animals

Animals were randomized into four groups of six animals each. The animals of Group I, II, and III were treated with topical application of cream A, B, and standard framycetin sulfate, respectively. For both excision and incision wound models, the animal groups were classified and treated in the same manner.

2.6. Incision wound model

Animals were anesthetized by ether before wound creation. The particular skin area was shaved using hair removal cream, Veet (manufactured by Reckitt Benckiser [India] Ltd., New Delhi) 1 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 1 cm apart using sterile absorbable sutures. Black surgical thread and curved needle number 19 were used for stitching the wound.

Table 2

Effect of topical application of cream A, B, and framycetin sulfate cream on percent wound contraction and epithelialization period of excision wound in diabetic rat model.

Groups	Days after wounding					Epithelialization period (days)
	4	8	12	16	20	
Percentage of wound contraction						
I	48.70 ± 1.76	66.67 ± 4.27	88.37 ± 0.83*	95.43 ± 0.53*	100	19.17 ± 0.31
II	48.47 ± 2.36	77.03 ± 3.09	92.0 ± 0.59**	96.60 ± 1.16**	100	17.00 ± 0.37***
III	27.20 ± 3.29	63.33 ± 3.81	82.90 ± 2.26	92.00 ± 0.85	100	20.50 ± 0.43

Data were expressed as means ± SEM for six rats in each group. The treated groups were compared by one-way ANOVA with post-test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus standard. Group I: Cream A treated group, Group II: Cream B treated group, and Group III: Framycetin sulfate treated group, SEM: Standard error of mean.

Cream A, B, and standard (framycetin sulfate) were topically applied once a day. The sutures were then removed on the 8th day post-wounding, and the tensile strength of 10-day old wound was measured by tensiometer [22].

2.7. Excision wound model

Animals were anesthetized before wound creation by open mask method using ether. The particular skin area was shaved using hair removal cream (Veet) 1 day prior to the experiment. A full thickness of the excision wound of circular area (approximately 500 mm²), and 2 mm depth was made on the shaved back of the rats. The wound was left undressed to the open environment. Cream A, B, and standard (framycetin sulfate) were topically applied once a day, starting from day 0 until complete epithelialization. The parameters studied were percent 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 epithelialization was calculated as the number of days required for falling off of the dead tissue remnants without any residual raw wound [23].

2.8. Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). Comparisons between the groups were performed using one-way ANOVA with post-test on GraphPad Instat 3 statistical software. Differences were considered significant, if P value is < 0.05 , 0.01 , and 0.001 .

3. Results

3.1. Safety evaluation (skin irritation study)

There were no signs of redness and itching when cream A and B were applied on the shaved back of albino rats. The primary skin irritation index of the creams was calculated as 0.00. This indicates that cream A and B were found to be safe.

3.2. Incision wound model

The results were presented as mean weight in gram ± SEM required to open the sutured wounds [Table 1]. Tensile strength of the wound in the animals treated with cream B and framycetin sulfate cream was found to be significantly higher ($P < 0.001$ and $P < 0.01$, respectively) as compared to the tensile strength of the wounds in animals treated with cream A. Tensile strength of the wounds in the animals treated with cream B was found to be greater as compared to tensile strength of the wounds in animals

treated with cream A and framycetin sulfate cream. Thus, more weight and more strength were required to break the wound treated with cream B as compared to wound treated with cream A and framycetin sulfate indicating that cream B was the most effective among all three creams. Tensile strength of the animals treated with standard (framycetin sulfate) was found to be greater than tensile strength of the animals treated with cream A. This indicates that framycetin sulfate cream has faster wound healing rate than cream A.

3.3. Excision wound model

The effect of wound healing activity in this model was evaluated by determining the percent wound contraction and epithelialization period. The results in Table 2 are expressed as % wound contraction and epithelialization period (mean \pm SEM). The studies on excision wound healing model revealed that both the groups (cream A and B) showed decreased wound area from day 0 to day 20. On the 12th and 16th day, the % wound contraction of excision wounds treated with cream A and B (95.43 ± 0.53 and $96.60 \pm 1.16\%$, respectively) was found to be significantly higher ($P < 0.05$ and $P < 0.01$, respectively) than the wounds treated with framycetin sulfate cream ($92.00 \pm 0.85\%$). The complete healing of wound is seen when the eschar falls. Wounds treated with cream B healed faster (day 17) and the healing was statistically significant ($P < 0.001$) as compared to the wounds treated with framycetin sulfate (day 21).

The period of epithelialization of wounds treated with cream A was found to be low as compared to standard group. This indicates that cream A and B promotes epithelialization of wound faster than framycetin sulfate.

4. 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 [24]. 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 B in comparison with framycetin sulfate cream in diabetic wound models. The results of the present study showed that cream A and B possessed a definite pro-healing action.

The results indicated that both the creams (cream A and B) did not cause any skin reaction after examining at 24, 48, and 72 h. It can be concluded that both the creams did not cause any skin irritation and can be classified as non-irritant. Both the creams were found to be safe for topical application.

In incision wound, the increase in the tensile strength of skin in treated wounds may be due to increase in the collagen concentration and stabilization of fibers facilitating wound healing. The highest tensile strength of the wounded skin was observed in the animals treated with cream B.

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

The results of the present study showed that the cream B possessed significant wound healing activity than cream A due to the synergistic effects of Jatyadi Oil, *C. asiatica*, and other ingredients present in cream B. Asiaticoside isolated from *C. asiatica* showed promising wound healing activity in normal as well as in diabetic animals [25]. Jatyadi Oil 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 Oil was also evaluated scientifically by *in vivo* evaluation in rat using excision wound model [14].

5. Conclusion

Cream A and B were found to be non-irritant and safe for topical application. Both cream A (Ari's Wound Healing Cream) and cream B (Amarantha Wound Healing Cream) enhanced wound healing process in albino rats when tested by incision and excision of fresh and diabetic wound models. Thus, the prepared topical creams possess multifaceted properties in healing the wound.

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Nil.

Conflicts of interest

None declared.

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