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Research Article

# FORMULATION AND EVALUATION OF AN HERBAL CREAM FOR WOUND HEALING ACTIVITY

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**ABSTRACT**

Wounds are physical injuries that results in an opening or breaking of the skin. Proper healing of wounds is very essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin. Wound healing is a complex but generally orderly process. Sequential waves of specialized cell types first clear the inciting injury and then progressively build the scaffolding to fill in any resulting defect. Cream is defined as semisolid emulsions which may be oil in-water (o/w) or water- in- oil (w/o) type and these semisolid emulsions are meant for external applications. Presently whole world including the developed country recognized the importance of traditional medicine and encouraging the research on herbal or ethnomedicine as they safe and less toxic. In this study we have formulated an herbal cream (O/W type) satisfying almost all the pharmaceutical parameters which showed better tissue regeneration and healing capacity. The wound healing activity of herbal cream was experimentally evaluated by excision wound model. The experimental data of wound size area and histopathologcal study expressed that, healing in the cream treated group of animal was significant as compared to control group of animal.

**Keywords:** Wound Cream, Herb, O/W emulsion, Excision wound, Wound size.

## INTRODUCTION

Wound may be defined as a loss or breaking of cellular and anatomic or functional continuity of living tissue. It is produced by physical, chemical, thermal, microbial, or immunological damage to the tissue. Wound healing or wound repair is the body's natural process of regenerating dermal and epidermal tissue.

Healing requires the collaborative efforts of various tissues and cell lineages. It involves aggregation of platelets, clotting of blood, fibrin formation, and an inflammatory response to injury, alteration in the ground substances, angiogenesis and re-epithelialisation. Healing process is not complete until the disrupted surfaces are firmly knit by collagen [1-4].

Cream is defined as semisolid emulsions which are oil in-water (o/w) or water- in- oil (w/o) type and these semisolid emulsions are intended for external applications. Creams are often composed of two phases. Oil-in-water (o/w) emulsions are most useful as water-washable bases, whereas water-in-oil (w/o) emulsions are emollient and cleansing agents. An emulsifying agent is used to disperse the aqueous phase in the oily phase or vice versa [5-8].

World Health Organization (WHO) as well our country has been promoting traditional medicine because they are less expensive, easily available and comprehensive, especially in developing countries [3, 5].

It is also true that eight percent of the world's population relies on medicinal plants for their primary health care. Whole world including the developed country recognized the importance of traditional medicine and has treatment strategies, guidelines and standard for ethnomedicine [6, 9].

Although various types of cream is considered for wound healing but these are still appears to be limited in rate of tissue regeneration. Hence after a depth review regarding pathogenesis as well as different traditional and alternative therapy for wound healing, we have taken up the project to develop and formulate an herbal cream which will be effective and has better rate of tissue regeneration [2, 3].. The herbal cream that is planned to be formulated for wound healing will be oil/water (O/W)emulsion type which will be less oily, less greasy and less sticky in nature so that patient compliance is more and will be beneficial for all kind of people in our society.

After thorough review of Ayurvedic and Homeopathic system of medicine we have selected following herbs to formulate the cream for wound healing action –

- *Panax ginseng (*promotes blood circulation, skin warming) [10],
- *Aloe vera (regenerate tissue)* [11, 12],
- *Calendula officinalis (*heal ulceration) [13],
- *Clerodendrum indicum (antimicrobial action)* [14, 15],
- *Arnica Montana,* (reduce pain due to injury) [16],
- Rose hip oil *(*rich source of vitamin C) [17, 18].

In this we have made an attempt to formulate most complete herbal cream that contains herbs which will satisfy almost all the mechanism to heal a wound effectively.

## MATERIALS AND METHODS

### MATERIALS

#### Collection of plant materials

The dried crude drugs of *Calendula officinalis*, *Arnica Montana and* extracted powder of *Aloe vera* were collected from API supplier, Kolkata. The fresh leaves of *Clerodendrum indicum* was collected from North Eastern Development Finance Corporation Ltd. (NEDFI), Khetri, Assam. The dried root of *Panax ginseng* was collected from B. S. Trading, Kolkata. Rose hip oil was collected from Katyani Exports, New Delhi.

#### Chemicals and Reagents

The chemicals used during the experiments were of analytical grade. Lanolin (Burgoyne Urbidges & Co), White petrolatum (Yarrowchem Products), Tween 60, Stearic acid (Himedia Lab), Mineral oil, Triethanolamine, Propylene Glycol (Merck Lab), Betadine; Povidone-iodine IP 5%w/w, (Win-Medicare) etc were used.

#### Instruments

Freeze Dryer (PIRANI; Kolkata), Homogenizer, Centrifuge (REMI), Viscometer (Brookfield DV-E viscometer), Digital Balance (Denver Instrument), Digital $p^H$ meter (Systronics) etc were used.

### METHODS

#### Extraction of plant materials

The extractions of crude drugs were carried out by Simple Maceration method using water (aqueous extract) as menstruum with occasional stirring. Liquid mixture is then pressed and filtered

to get a clear liquid extract. The clear liquid is then subjected to freeze drying in order to get a solid mass [19, 20].

**Formulation of herbal cream (O/W emulsion)**

Ingredient of oil phase (A) was melted in a beaker by using water bath on constant stirring. Components of aqueous phase (B) were mixed together and warmed to about same temperature of oil phase (up to $70^0$ C). The preservative methyl paraben and concentrated aqueous extract of the plants were added into aqueous phase and heated. Then oil phase was added to water phase little by little on constant stirring and perfume was added to it when the temperature was $35^0$C - $40^0$ C. Six different formulations (**F**1-**F**6) were prepared by using varying concentration of aqueous extract, stearic acid and liquid paraffin [8, 21, 22].

**Pharmaceutical evaluation of cream**

The formulations or creams were evaluated for different pharmaceutical parameters: such as Type of emulsion (Dye method and Dilution method), Homogeneity, Appearance, After feel, Type of smear, Removal, Creaming or coalescence, Globule size analysis, pH (Digital $p^H$ meter, systronics), Extrudability, Viscosity, (Brookfield Viscometer), stability testing and Spreadability. The best formulation was selected on basis of their pharmaceutical parameters and evaluated for wound healing activity [7, 23-25].

***In vivo* evaluation of herbal cream**

**Skin irritation test**

The cream was evaluated for primary skin irritation test on experimental animals (shaved back of the rats) to evaluate the safety of cream [3, 5, 6,].

**Evaluation of wound healing activity**

The healthy Wistar albino rats of either sex, weighing 150–200 gm, were housed under standard environmental conditions of temperature, humidity (25 ± 0.50˚C) and 12 hr light/dark cycle. The animals were fed with standard pellet diet and water *ad libitum*. The experiment was conducted in accordance to the protocol approved by Institute Animal Ethics Committee (IAEC), Girijananda Chowdhury Institute of Pharmaceutical sciences. Guwahati, Assam. (Registration no- 1372/c/10/CPCSEA).

The rats were divided into three groups namely control (base cream treated group/without extract), standard (Povidone-Iodine treated group/Betadine) and test (formulated herbal cream) group. On the day of experiments rats were anesthetized by administering ketamine (50 mg/kg i.p.). A full thickness of the excision wound with circular area of 176 $mm^2$ (width 1.5 cm and depth 0.2 cm) was made on the shaved back (dorsal thoracic region) of the rats. The wounding day was considered as day 0. The wounds were treated with topical application of the cream once daily till complete epithelisation. The wounds were monitored and the area of wound size was measured on 3, 6, 9, 12, 15 & $18^{th}$ of post-wounding day. The wound size area and mean % wound closure/contraction were noted. The results were reported in Mean±SEM and the data was analyzed using ANOVA [2, 3, 26-29].

The percentage (%) of wound closure was calculated using the following formula:

$$\%\ \text{of wound closure} = \frac{\text{Wound Area on Day '0'} - \text{Wound Area on Day 'n'}}{\text{Wound Area on Day '0'}} \text{X100}$$

Where *n* =number of days.

**Histopathological studies of wounded skin**

At the day $18^{th}$ the experiment was terminated and tissue of wound area was removed from the surviving animals for histopathological examination. Sample tissues were fixed in 10% formalin and were embedded in paraffin wax. Serial sections (5µm thickness) of paraffin embedded tissues were cut. The tissues stained by haematoxylin and eosin, (HE staining) and after that they were examined by electronic microscope [29-31].

**RESULTS AND DISCUSSION**

**Pharmaceutical evaluation of herbal cream**

**F**6 formulation or cream was found to be best and satisfactory compared to all other formulations. It had light brown appearance, gave a cool and smooth feel on application which was maintained after tested the stability study. Stability was determined by exposing the formulation to various temperatures such as 4°C, 27°C & 37°C for specified period. The $p^H$ of the formulation was found to be 6.60 which is good for skin ($p^H$=6.8). The creams also showed good spreadability (16.17 g.cm/sec) when measured using slides. Lesser the time taken for separation of two slides, resultant the better spreadability. Spreadability was calculated by using the formula. (S= M.L/T). Where S= spreadability, M= Weight tied to upper slide, L= Length of glass slides and T= Time taken to separate the slides completely from each other. After application of the cream the type of smear formed on the skin was found to be non-greasy and easily removed on washing with tap water. The viscosity of the creams was found to be 17,650 cps, with 25 rpm, which indicates that the prepared cream was easily spreadable with small amount of shear.

**Table 1: Final composition of herbal cream (formulation F6)**

| Ingredients | Formulation %w/w in grams |
|---|---|
| Aq. Extract of *Panax ginseng* | 5.0 |
| Aq. Extract of *Calendula officinalis* | 5.0 |
| Aq. Extract of *Arnica Montana* | 2.0 |
| Aq. Extract of *Clerodendrum indicum* | 1.0 |
| *Aloe vera* | 3.0 |
| Rose hip oil | 4.0 |
| White petrolatum | 0.8 |
| Liquid paraffin | 8.3 |
| Lanolin | 0.8 |
| Stearic acid | 16.7 |
| Propylene glycol | 3.5 |
| Triethanolamine | 1.0 |
| Tween 60 | 5.0 |
| Methyl paraben | 0.1 |
| Water | q.s |

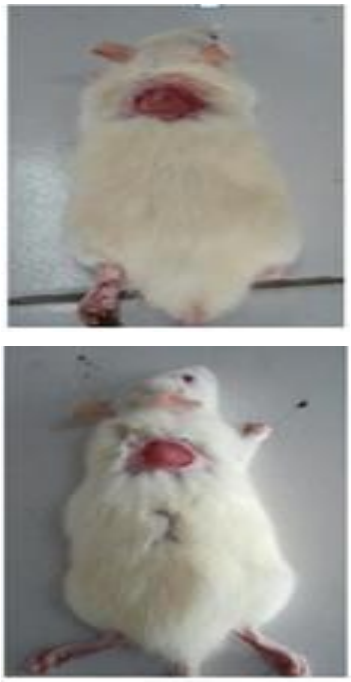
**Skin irritation test**

This test was conducted to evaluate the irritation caused by the prepared cream on the intact skin of animals. The results showed that the formulation (**F**6) was devoid of any primary skin irritation or sensation or erythema, or edema even after 48 hrs of application on the rat skin. None of the animal showed any skin reaction.

**Wound healing activity**

The results of wound healing activity by excision wound model are presented in **Table 2 and figure 3**. The values of wound area are presented in $mm^2$ at 0, 3, 6, 9, 12, 15 and $18^{th}$ days. The results indicate that standard cream and test herbal cream both significantly ($P < 0.01$) reduces the wound area as compared to the control group.

**Table 3 and figure 4** represents percentage (%) wound healing (wound contraction) at 9 and $18^{th}$ days for control, standard and the test groups. It is observed that wound contracting rate of animals treated with herbal cream and standard cream significantly higher ($P < 0.01$) on days 9 and $18^{th}$ as compared to the control group.



**Control Standard Test**

**Fig. 1: Wound at day 0**

**Control Standard Test**

**Fig. 2: Wound healing after day 15**

**Table 2: Effect of herbal cream on Wound size at different days interval**

| Groups | Wound size area in mm² (mean ± SEM) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 day | 3 day | 6 day | 9 day | 12 day | 15 day | 18 day |
| Control | 176.60 ± 0.93 | 162.45 ± 0.83 | 113.00 ± 0.85 | 63.58 ± 0.65 | 38.46 ± 0.93 | 19.62 ± 0.95 | 10.36 ± 0.33 |
| Standard | 172.44 ± 0.83 | 136.66 ± 0.85 | 66.44 ± 0.89 | 30.13 ± 0.63 | 13.83 ± 0.91 | 3.10 ± 0.33 | 0.26 ± 0.05** |
| Test | 174.24 ± 0.85 | 136.78 ± 1.21 | 72.34 ± 0.89 | 30.17 ± 0.63 | 16.62 ± 1.06 | 3.20 ± 0.35 | 0.28 ± 0.05** |

The treated, standard groups are compared with the control group.

*** $P < 0.001$.

** $P < 0.01$.

* $P < 0.05$.

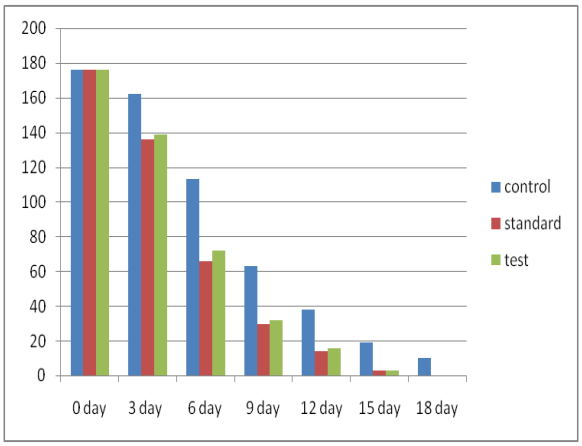



**Fig. 3: Wound size area (mm²) at differents days interval**

**Table 3: Effect of herbal cream on % wound contraction of excision wound at different days interval**

| | % Wound contraction of excision wound (mean ± SEM) | | |
|---|---|---|---|
| | 0 day | 9 day | 18 day |
| Control | 00 | 39.96 ± 3.83 | 75.33 ± 3.85 |
| Standard | 00 | 58.33 ± 1.66 | 95.53 ± 3.85 ** |
| Test | 00 | 57.66 ± 2.33 | 95.53 ± 3.85 ** |

The treated groups and standard groups are compared with the control group.

*** $P < 0.001$.

** $P < 0.01$.

* $P < 0.05$.

**Histopathological study**

The characteristics observed during histopathological examination were the proliferation of fibroblasts, granulation tissue, collagen fibre and tissue remodelling etc. Compared to control groups the above mentioned parameters were more conclusive and decisive in case of both standard and test group. This histopathological observation also provided additional evidence for the experimental wound healing activity. The details of histopathological data are given in **figure 5, 6 and 7** below.

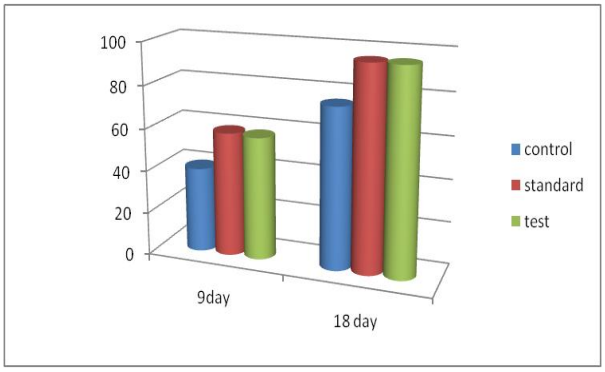



**Fig. 4: % Wound contraction of excision wound at different days interval**

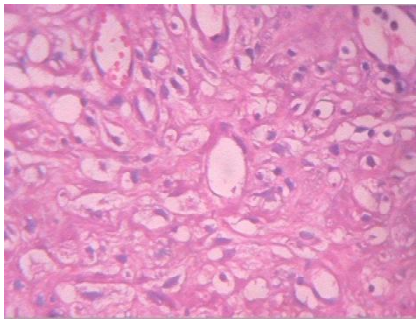


**Fig. 5: Histological image of control at HEx20**

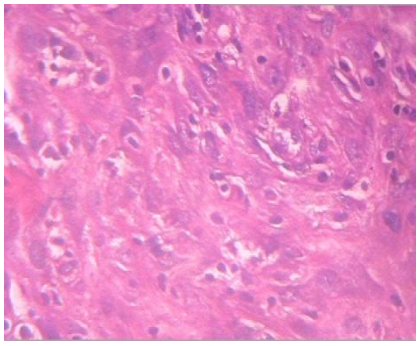


**Fig. 6: Histological image of standard at HEx20**

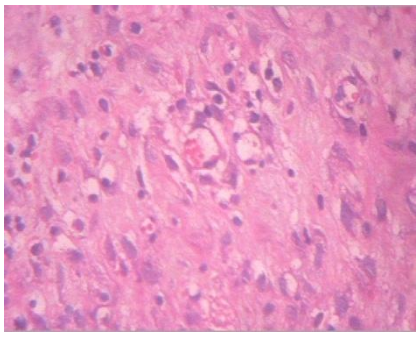


**Fig. 7: Histological image of test at HEx20**

## CONCLUSION

The prepared cream was pleasant, coolant, easily spreadable and washable thereby there is a chance of increased the patient compliance. Formulated cream significantly promotes wound healing than control or non-medicated group. The activity may be mainly due to free radical scavenging activity, antioxidant activity and anti-inflammatory effect of the polyphenols and flavonoids present in the different extract. Some of herbs reported to act by supplying Vit. C and by promoting tissue regeneration. However, further depth and structured study, would be beneficial to assess its usefulness and mechanisms more exactly. This study can be helpful for upcoming researchers to select these herbs for the formulation and evaluation of other cosmetic applications which can be claimed for their efficacy with scientific data.

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