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Design and Formulation of *Tridax procumbens* based Polyherbal Cream for Wound Healing Potential

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ABSTRACT

The present research has been undertaken with the aim to design, formulate and evaluate the polyherbal Tridax procumbens based cream comprising of Aloe vera, Marigold, Henna, Papaya and Neem. The cream was evaluated for pharmaceutical parameters & wound healing activity. The cream was formulated using accurately weighed amount of drug extract along with base and other suitable additives. The pH of all the formulations were checked and found to be compatible with the normal pH range of the skin. The formulation showed good spreadability, homogeneity, consistency, there was no change in the appearance and no phase separation was noticed. The cream was evaluated for wound healing activity as period of contraction and tensile strength using excision wound model. Albino wistar rats were divided into 5 groups where betadine ointment was used as reference standard, one served as positive control (ointment base), one negative control and the other two as treated groups. In the model, the rate of wound contraction was accused as healing parameter at every third day. The result showed that topical application of cream increases the percentage of wound contraction and decreases epithelization time in treatment group as compared to other groups which may be due to additive activity of the phytoconstituents present in the extract and hence, may be used as a potential herbal formulation for wound healing.

Keywords: Polyherbal formulation, Excision wound, *Tridax procumbens, Curcuma longa, Calendula officinalis, Azadiracta indica, lawsonia inermis, Carica Papaya*.

INTRODUCTION

Wound is a physical trauma where the skin is torn, cut or punctured. On exposure to air, microorganisms enter the wound which leads to wound contamination and finally development of infection [1]. It is a process that is fundamentally a connective tissue response. Initial stage of this process involves an acute inflammatory phase followed by synthesis of collagen and other extracellular macromolecules that helps in the formation of a scar. This intricate process is initiated in response to an injury restores the function and integrity of damaged tissues [2].

Herbal therapy predominates in traditional medicine as well as in alternative medicine practiced in the developing and the developed countries. The widespread interest in drugs derived from plants is because of the belief that plants are safe and dependable, and with lesser side effects. Review of literature reveals that traditional plant drugs are beneficial for several skin related problems and for wound healing [3]. World Health Organization (WHO) as well as our country has been promoting use of traditional medicine because they are less expensive, easily available and comprehensive, especially in developing countries [4,5]. Although various types of creams are considered for wound healing but these still appears to be limited in rate of tissue regeneration [4,6]. Creams intended for external applications are often composed of two phases. An emulsifying agent is used, which form film around the globules of disperse phase, so that a stable emulsion is produced [7-8]. The objective of the present study was to formulate and evaluate polyherbal cream that contains herbs which will satisfy almost all the mechanism to heal a wound effectively.

MATERIALS AND METHODS

Collection of plant materials

The plant species comprising of Aloe, Marigold, Neem, Heena, Papaya, and Ghamra were collected from the Garden, AKS University, Satna, M.P., India**.** The taxonomic identities of plants were confirmed by DRI, Chitrakoot, Satna, M.P., India. They were washed with tap water and shade dried. The dried leaves were powdered using mechanical grinder and were stored in well closed container at 4°C. The powders were used for the further process.

Preparation of extracts from plant leaves

Each powdered plant part was subjected to Soxhlation process using the various solvents. 30 gm of powdered samples and 300 ml of solvents were used for each extracts. The extracts were collected and concentrated using Rotary Vacuum Evaporator. The crude semi-solid extracts were collected and stored in small vial. The extracts were stored at 4°C until further use for various evaluations.

Instruments

Digital Balance (Denver Instrument), Digital pH meter (Systronics), Scale, Surgical blades (No.18), Ketamine (Anesthetics), Betadine, Annie French, (Hair remover cream), Forceps, Cotton.

Chemicals and Reagents

White wax, white petroleum, PEG 300, Cetostearyl alcohol, methyl paraben, propyl paraben.

Composition of Ointment base

The ointment base was used as a carrier to deliver the drug to the wound. A suitable base should be needed to formulate herbal extract. The base must be compatible with the extracts to be incorporated into it. In this respect, following points must be considered.

About 2-4gm of ointment may be lost in the compounding process. To compensate for this loss, an excess of the ointment was prepared. Some general rules might be to be add 10% or 3gm to the prescribed amount [9].

Table No:1 Ointment Base composition (50gm)

S. No.	Name of Ingredients	Quantity (gm)
1.	White wax	12.50
2.	White petroleum	12.50
3.	PEG 300	20.00
4.	Cetostearyl alcohol	12.50
5.	Methyl paraben	0.025
6.	Propyl paraben	0.015

Preparation of Ointment base

White wax was melted on hot plate, the temperature of which was not exceeded beyond 70-75^{0}C. When the wax was completely melted, white petroleum was added and allowed the entire mixture to remain on the hot plate until liquefied. Following liquefaction, removed from heat and allowed the mixture to congeal. The mixture was stirred until it began to congeal.

Preparation of polyherbal cream

The semi dried extracts were used for the preparation of cream. The polyherbal formulations were prepared by using an ointment base. Standard trituration method was used where solid fats were melted and mixed. The required quantity of the ointment base was weighed and melted at a temperature of about 70°C using hot plate. The designated quantity of the extract was respectively added to the melted base at 40°C and then mixed. The preparation stirred gently and continuously until a homogeneous dispersion was obtained.

Table No:2 Formula for formulation

S.No.	Name of Extract	Formulation 1 Quantity (%)	Formulation 2 Quantity (%)
1.	Ghamra (Tridex Procumbens)	4%	2%
2.	Papaya	1%	1%
3.	Heena	1.5%	1%
4.	Neem	1%	1%
5.	Marigold	1%	1%
6.	Aloe vera	1%	0.5%
7.	Ointment base	93%	91.5%

Standardization of the polyherbal formulation

The polyherbal formulations were evaluated by the following physicochemical parameters:

The pH meter was calibrated using standard buffer solution. About 0.5 gm of the cream was weighed and dissolved in 50.0 ml of distilled water and its pH was measured. The scarlet red dye was mixed with the cream. A drop of the cream was placed on a microscopic slide, cover slip was placed and was examined under a microscope. If the disperse occured in w/o type cream i.e. the disperse globules appear colorless in the red ground. Globules appeared red the ground colorless. The cream was o/w type i.e reverse condition. The formulations were tested for the homogeneity by visual appearance and by touch. The appearance of the cream was judged by its color, pearlscence, roughness and graded**.** Emolliency, slipperiness and amount of residue left after the application of fixed amount of cream was checked**.** After application of cream, the type of film or smear formed on the skin were checked.

The spread ability was expressed in terms of times in seconds taken by two slides to slip off from ointment placed in between the slides under the direction of certain load. Spread ability 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

The ease of removal of the cream applied was examined by washing the applied part with tap water**.** The cream was evaluated for primary skin irritation test on experimental animals (shaved back of the rats) to evaluate the safety of cream [10-13].

Experimental animals

The experiment was carried out on Wistar albino rats of 4 months, of both sexes, weighing between 140 to 180 gm. In the experiment, a total of 30 rats were used. Study protocol was approved by Institutional Animal Ethics Committee and conducted as per CPCSEA guidelines.

The rats were divided into 5 groups comprising of 6 animals in each group as follows:
Group I: Left untreated and considered as control.
Group II: Served as reference, treated with Betadine (5% Ointment) once daily
Group III: Treated with Sample-1 once daily
Group IV: Treated with Sample-2 once daily
Group V: Treated with Sample-3 once daily, ointment base

Excision wound model

The wound was created using excision method. For this, hairs were removed from the posterior sides of rats using hair remover cream. The wound of about 10 mm diameter was measured with sterile scale and this area was marked with a marker pen. The rats were anesthetized with ketamine (50mg/kg i.p.). After 15 minutes of anaesthesia, the marked area of skin was excised with the help of surgical blade no. 18 and forceps. The skin was removed after creating the wound. The unknown samples and Betadine were applied, starting from the next day of the operation, till complete epithelialisation time. The parameters studied were wound closure and epithelialisation time. The wound was measured using transparency paper, a marker, scale and area was calculated at 0, 3, 6, 9, 12 and 15 days. The period of epithelialisation was calculated as the number of days required for falling of the dead tissue remnants of the wound without any residual raw wound [14-15].

The percentage wound contraction was determined using the following formula:

$$\text{Percentage of wound contraction} = \frac{\text{Initial wound size} - \text{Specific day wound size}}{\text{Initial wound size}} \times 100$$

Statistical Analysis

All the values were expressed as mean±standard error of mean (S.E.M.) and analyzed by one way ANOVA and post hoc Tukey multiple comparison test by employing statistical software, Graph Pad InStat 3. Differences between groups were considered statistically significant at $P < 0.05$ levels.

RESULTS AND DISCUSSION

The pH of the cream was found in the range which is good for skin pH. Both the formulations of cream showed pH nearer to skin as required i.e. pH of F1-6.5, F2-6.6. The formulation F1 and F2 showed no redness, edema, inflammation and irritation during irritancy studies. These formulations were safe to use for skin. The dye confirmed

that both formulations were w/o type emulsion cream. All formulations produced uniform distribution of extracts in cream. This was confirmed by visual appearance and by touch. The cream as greenish brown in colour. When formulation were kept for long time, it found that no change in colour of cream. Emolliency, slipperiness and certain amount of residue left after the application of fixed amount of cream was found. After application of cream of F1 and F2, the type of smear formed on the skin were greasy. The cream of F1 and F2 applied on skin were not easily removed with tap water (Table No:3). The 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**1 and F2) 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.

Table:3 Physicochemical properties of the formulation

S.NO.	Parameters	observation
1.	Temperature	Room temperature
2.	Formulation	w/o type cream
3.	pH	6.55 (F1), 6.67 (F2)
4.	Homogeneity	Good
5.	Appearance	Greenish Brown color
6.	Spread ability	Satisfactory
7.	After feel	Emollient
8.	Type of Smear	Greasy
9.	Removal	Non removal

Wound Healing activity

Wound healing involves a cascade of events characterized by completion of biological processes in a certain order and a certain time frame. These events represent the restructuring of the damaged tissue in an attempt to restore as normal a condition as is possible. The natural response of a living organism is to repair the wounds in the shortest time possible and to re-establish the normal continuum of the structures [16].

The study on excision wound healing model revealed that all the groups showed day to day decrease in wound area. However, on 15th post wounding day, untreated animals (group-I) showed 83.53% of wound contraction whereas group-II standard group animals, showed that of 100% and the formula-1 and formula-2 group-III & IV also exhibited 95.31% & 91.07% wound contraction respectively. Formula-3 as ointment base treated group showed wound contraction similar to control. When compared with the standard, the activity of the formula-1 and formula-2 treated group was found to be of significant value. It was also observed that reducing the epitheliazation period of formula-1 (15.83) and formula-2 (16.66) group in comparison to control group (Table- 1). The time required for complete epitheliazation of the excision wound is an important parameter to assess the wound healing process. Results of above study revealed that formula 1 & formula -2 have almost equal potential to treat excision wound while formula- 3 was not showed any treatment.

Table:4- Effect of Unknown samples on excision wound model.

Groups	**% Wound contraction**					**Epitheliazation period (Days)**
	3th day	**6 th day**	**9th day**	**12th day**	**15th day**	
Control	18.61±2.31	30.20±4.28	46.96±1.43	71.54±4.81	83.53±3.29	17.83±0.3
Standard	40.36±3.38a**	48.03±1.35 a**	75.7±4.78 a***	92.02±3.66 a**	100.0±0.0a**	13.5±0.22a***
F-1	34.66±5.11a*	45.89±1.35 a**	74.75±4.46 a***	90.62±2.96 a*	95.31±2.96	15.83±0.3a***,b***
F-2	33.18±4.73	44.82±1.07 a*	70.59±4.31 a***	86.38±2.95	91.07±4.08	16.66±0.33 b***
F-3	19.02±2.43	38.04±4.73	48.03±1.35b***	73.81±4.07b*	88.72±3.71	17.0±0.25 b***

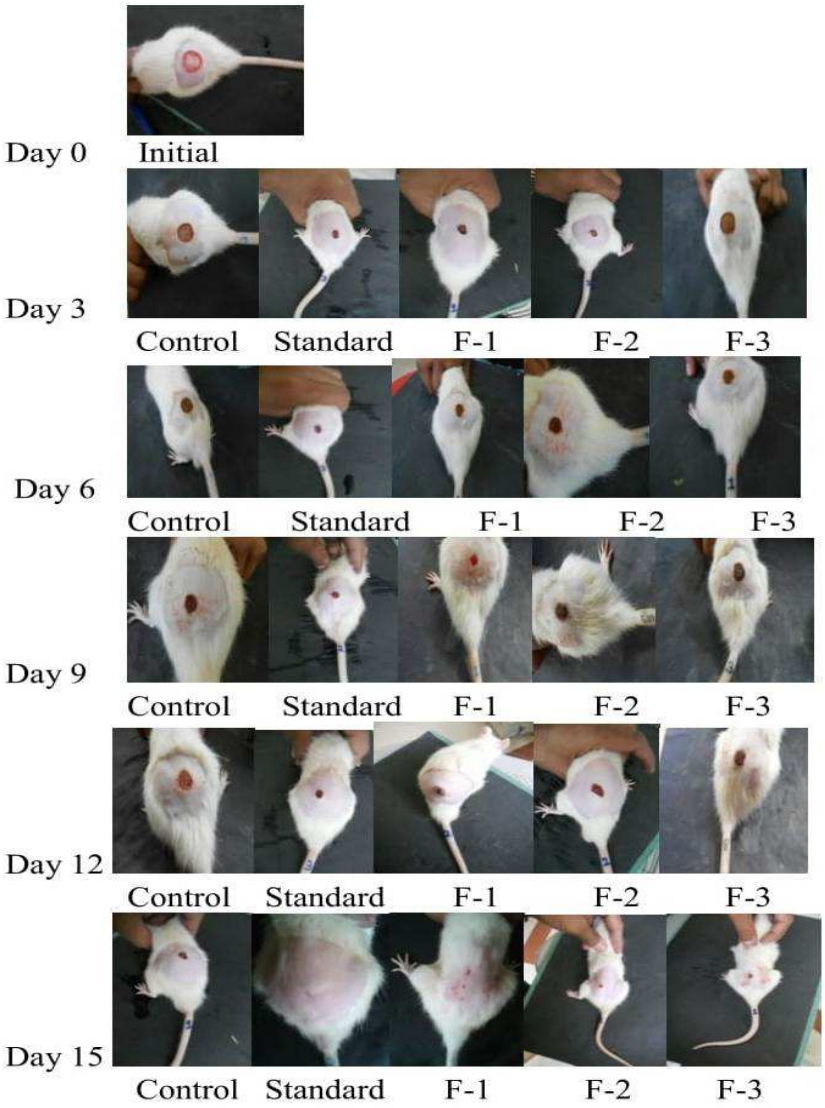

Figure 1: wound of control, standard, formula 1,2,3 (ointment base) at day 0, 3rd, 6th, 9th, 12th, 15th day

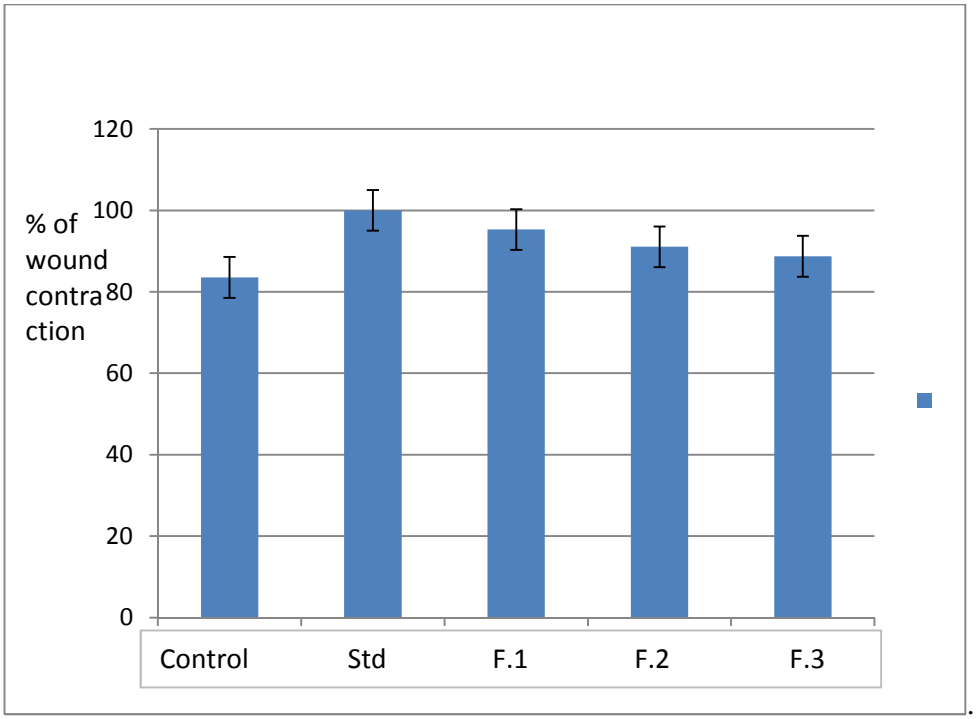

Figure showing % wound contraction graph

Figure 2: Effect of the polyherbal formulation in excision wound model

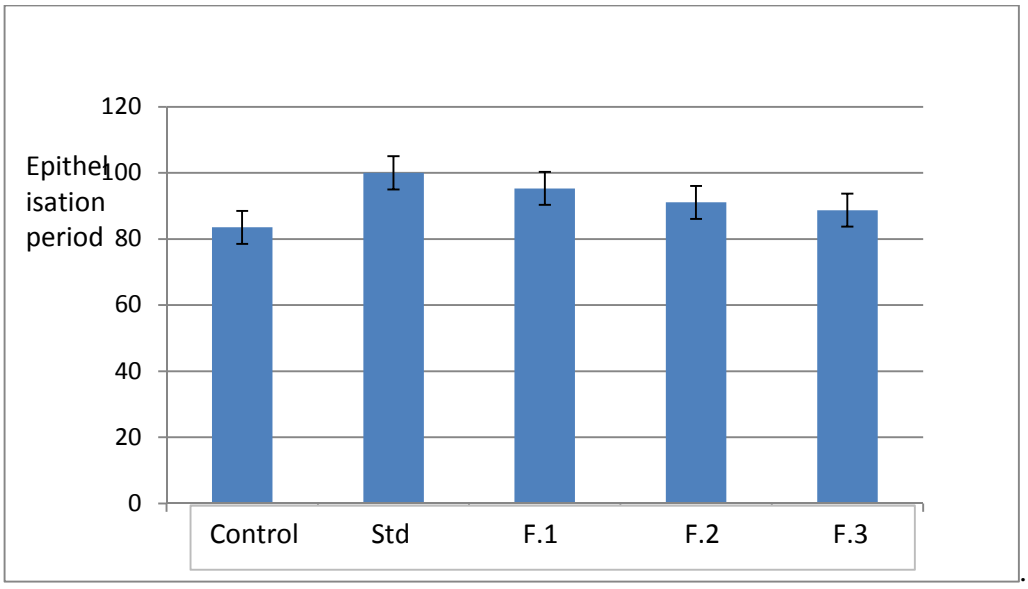

Figure 3: showing epithelisation period for the sample under study

CONCLUSION

On the basis of the results obtained in the present study, the combination of Aloe, Marigold, Neem, Heena, Papaya, and Ghamra formulated as polyherbal cream accelerated the healing process by enhancing collagen formation and increasing the breaking strength of the healed wounds. The potent activity of the polyherbal formulation can be attributed to the phyto-constituents present in the formulation which may be due to additive activity of the phytoconstituents present in the extract to enhance the wound healing effect.

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