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Research Article

Evaluation of Wound Healing Activity of Polyherbal Skin Care Cream

Abhinay Kumar Dwivedi*, Hemant Kumar Sharma

Sri Satya Sai University of Technology and Medical Sciences, Sehore, M.P., India-466001

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*Address for Correspondence:

Abhinay Kumar Dwivedi, Sri Satya Sai University of Technology and Medical Sciences, Sehore, M.P., India-466001

Abstract

Plants have long history of use to cure and prevention of various human ailments. In the present study *Azadirachta indica, Ocimum sanctum, Centella asiatica and Hibiscus rosa sinensis* extracts have been used to formulate skin care cream with varied proportion of individual extract. The formulations F3 and F4 have been selected for evaluation of wound healing study on the basis of preliminary evaluation results of parameter evaluated for Polyherbal cream. The formulation F3 and F4 were compared with negative control and standard treated group. It was found that the formulation F4 has greater wound contraction and efficiently reduced the time of epithelization campared to negative control with significant level P<0.050 and P>0.001, results indicated that the formulation F4 could be used as effective agent in wound healing.

Keywords: Herbal cream, Wound healing, Flavonoids, Phytochemical

INTRODUCTION

Wound is defined as the disruption of the anatomic and cellular continuity of tissue caused by chemical, physical, thermal, microbial, or immunological injury to the tissue. Wound healing processes consist of integrated cellular and biochemical cascades leading to reestablishment of structural and functional integrity of the damaged tissue.[1,2] Wound healing is a natural body reaction leading to the restoration of structural and functional integrity of injured tissues. It is a complex and dynamic process of replacing devitalized and missing cellular structures and tissue layers. The wound healing is a normal biological process, and it involves four complex steps: homeostasis/coagulation; inflammation, migration, and proliferation; reepithelialization and restoration. Each phase of the wound healing process is influenced by a series of mediators such as platelets and cytokines, inflammatory cells, cellular and extracellular matrix, proteinases, growth factors, and inhibitors. In the present study in vivo wound healing activity of the formulation polyherbal cream has been studied using excision wound model.[3,4] Various herbal products have been used in management and treatment of wounds over the years.[5] Many phytoconstituents such as flavonoids and polyphenols can heal wounds, in addition to having antioxidant, anti-inflammatory, and antimicrobial actions that contribute to wound healing processes and are generally easily accessible and have limited side effects.[6]

MATERIALS AND METHODS

Analytical grade chemicals have been used for the purpose of study, all the chemicals procured from Central Drug House (P) LTD. New Delhi, The glass wares used in the study have borosilicate and ASGI mark.

Collection and Processing of Plant Material

The leaves of *Ocimum sanctum, Azadirachta indica, Centella asiatica* and *Hibiscus rosa sinensis has* been procured from the botanical garden Bansal College of Pharmacy, Bhopal M.P. India, in the month of march april. Plant samples were authenticated by Dr. Suman Mishra, Botany Scientist, MFP-PARC, Bhopal, MP. All collected plant materials were washed with tap water to remove debris and dirt then shade dried for seven days, The dried plant material then grounded using electric grinder, the powdered plant material the sieved through sieve no. 40 to get fine powder, The fine powder has been extracted with hydro alcoholic solvent.

Extraction of Plant Material

The hydro alcoholic extract of plant material has been prepared by soaking the plant material in 80% ethanol for seven days.100 g of each powdered plant material has been macerated with with occasional stirring, the extract were collected and filtered using whatman filter paper, the filtrate were used to concentrated under reduced pressure at the temperature 40°C by rotary evaporator.The concentrated extracts were placed in the desiccator to remove residual solvent.

Phytochemical analysis of prepared extracts

Phytochemical screening of hydroalcoholic extracts has been done to ensure the presence of phytoconstituents alkaloids, glycosides, carbohydrate, flavonoids, phenolic compound,

saponin and triterpenoids by chemical tests. The results have been shown in table 3.

Formulation of Cream

The plant extracts collected above have been used to formulate the cream by using suitable base the formulation and selection of base has been done on the basis of preliminary evaluation of base cream then selected base has been used to formulate the cream, varying proportion of extract mixture have been used to formulate F1 to F6 cream. All ingredients used in the formulation were weighed accurately. The cetyl alcohol, soya lecithin, stearic acid, was melted in a beaker and heated up to 75°C. The plant extract were dissolved in water then filtered. To the filtrate humectant glycerol was added and heated to 75°C. When the temperature of both oil and water phases reached up to 75°C. The hydrophilic aqueous phase was added slowly into hydrophobic oily phase with continuous stirring until the mixture gets cooled. Preservative sodium benzoate has been added when the mixture became cooled and left at room temperature to obtain the required product. The flavoring agent was added at the last to get desired flavour to herbal cream.[7,8] The compositions of the herbal cream are given in table 1.

Table 1: Ingredients and concentration used in formulations

Ingredients	Formula % w/w					
	F1	F2	F3	F4	F5	F6
Stearic acid	11	11	11	11	11	11
Cetyl alcohol	8	8	8	8	8	8
Soya lecithin	2.5	2.5	2.5	2.5	2.5	2.5
Glycerol	5	5	5	5	5	5
A.indica	1	0.5	2	1.5	2.5	3
O.sanctum	0.5	1	1.5	2	3	2.5
C. asiatica	1.5	2	0.5	1	1.5	2
H.sinensis	2	1.5	1	0.5	2	1.5
Sodium Benzoate	0.20	0.20	0.20	0.20	0.20	0.20
Rose water	7	7	7	7	7	7
Water, qs, 100	qs	qs	qs	qs	qs	qs

Evaluation of cream

The evaluation of herbal cream has been done to check the quality of prepared cream; the cream has been tested for homogeneity, appearance, spredibility, after feel, type of smear, $_{p}$H, viscosity, type of emulsion and wound healing effect. The physical parameters of herbal creams were studied on room temperature and accelerated temperature.[9,10]

Experimental protocol and animals

The study was carried out at Pinnacle Biomedical Research Institute (PBRI), Bhopal (Reg. No. 1824/PO/Rc Bi/S/15/CPCSEA). The protocol of the study has been approved Institutional Animal Ethics Committee (IAEC), Protocol approval reference number was PBRI/IAEC/10-09-22/011. Healthy albino wistar rats of either sex weighing 190 to 230 g were selected for the study. The animals were kept under 12:12 h day and light schedules with temperature between 18ºC to 20ºC. They were housed in large spacious hygienic cage during experimental period. Animals were allowed to free access to water and standard pellet diet up to the end of the study.

Wound healing activity

The animals were anesthetized with slight vapor inhalation of diethyl ether, and the hairs were removed from the dorsal thoracic region. Excision wounds of 200 mm^2 size and 1 mm depth were made by cutting out pieces of skin from the shaven area. The entire wound was left open. The animals were closely observed for any infection and those that showed any sign of infection were separated, excluded from study, and replaced. Then the sample (F3 and F4) and standard Povidone iodine ointment (5% w/w) were applied every day to the specified groups for 24 days. Wound areas were measured on days 2, 4, 8, 12, 16, 18, 20 and 24 for all groups, using a transparency sheet and a permanent marker. The day the scar fell off, after wounding without any residual raw wound, was considered as the day of epithelization. This model was used to monitor the rate of wound contraction and epithelization.[11-14]

The percentage wound contraction calculated as:

% of wound contraction =

$$\frac{\text{Wound Area on 0 day - Wound Area on Test day}}{\text{Wound Area on 0 day}} \times 100$$

Epithelialization period

It was evaluated by noting the number of days required for the scar to fall off from the wound surface exclusive of leaving a raw wound behind. The falling of the scar from around the wound was taken as the end point of complete epithelization and the total days required for this were taken as the period of epithelization.

RESULTS AND DISCUSSION

Evaluation of cream

The formulated poly herbal cream was found satisfactory on all preliminary evaluated parameter of Easy removal, Homogeneity, Appearance, Spredibility, After feel, Type of smear Removal, Viscosity ,Type of emulsion, the results of evaluation were shown in table 2.

Table 2: Parameter evaluated at room temperature

F	Parameter at Room Temperature								
	H	A	S	AF	TS	R	pH	V	TE
F1	**	NC	**	E	NG	ER	6.5	16092.72±0.03	o/w
F2	***	NC	**	E	NG	ER	6.3	16023.18±0.06	o/w
F3	***	NC	***	E	NG	ER	6.2	16037.61±0.02	o/w
F4	***	NC	***	E	NG	ER	5.9	16041.47±0.05	o/w
F5	**	NC	***	E	NG	ER	6.3	16101.53±0.04	o/w
F6	***	NC	***	E	NG	ER	6.5	16113.29±0.03	o/w

***: Excellent **: Good *: NC: No change in colour, Satisfactory, E: Emollient NG: Non greasy ES: Easy removal H-Homogeneity, A-Appearance, S-Spredibility, AF-After feel, TS-Type of smear ,R-Removal, V-Viscosity ,TE-Type of emulsion, F-Formulation

Phytochemicals analysis

Phytochemicals analysis of hydroalcoholic extracts revealed the presence of alkaloids, carbohydrate, flavonoids, polyphenols, tannins, steroids and triterpenoids in *Azadirachta indica* and *Ocimum sanctum* shown the presence of flavonoids, saponin and triterpenoids polyphenols. *Centella asiatica* showed presence of alkaloids, carbohydrate, flavonoids, polyphenols, tannins, saponin and triterpenoids and *Hibiscus rosa sinensis* alkaloids, glycosides, flavonoids, polyphenols, tannins, saponin.

Table 3: Phytochemical analysis of hydro alcoholic extracts

Phytoconstituents	*Azadirachta indica*	*Ocimum sanctum*	*Centella asiatica*	*Hibiscus rosa sinensis*
Alkaloids	+	-	+	+
Glycosides	-	-	-	+
Carbohydrate	+	-	+	-
Flavonoid	+	+	+	+
Polyphenols	+	+	+	+
Tannins	+	-	+	+
Saponins	-	+	+	+
Steroids	+	-	-	-
Triterpenoids	+	+	+	-

+ = Present, - = Absent

Wound contraction

In the excision wound model, the F4 formulation showed statistically significant wound area contraction compared to the Negative Control. The higher wound contraction rate of the F4 was possibly due to macrophage cell proliferation. Both F3 and F4 formulations prepared showed fast wound contraction and reduced epithelization period. The complete wound closure was observed in standard and F4 formulation treated groups within 16 days.

Table 4: Wound contraction in mm

Sr.no	Group	Time in days							
		2	4	8	12	16	18	20	24
1	Negative control	177.00± 8.579	160.83± 5.879	137.83± 7.305	117.17± 4.997	68.67± 5.922	13.50± 1.871	4.00± 6.229	0±0
2	Standard (Povidone iodine 5%)	173.00± 11.576^{NS}	150.33± 17.108^{NS}	97.67± 16.120*	50.17± 7.360*	2.33± 2.066*	0±0*	0±0^{NS}	0±0
3	F3	178.00± 9.716^{NS}	148.33± 9.352^{NS}	99.83± 11.444*	49.00± 7.797*	17.50± 4.183*	0.33± 0.516*	0±0^{NS}	0±0
4	F4	170.17± 6.242^{NS}	142.50± 8.735*	92.83± 13.934*	43.67± 8.664*	1.67± 1.211*	0±0*	0±0^{NS}	0±0

Values are expressed as MEAN±SD at n=6, One-way ANOVA followed by Bonferroni test, *P<0.050 and ^{NS}P>0.001 compared to the negative control

Table 5: Percentage wound contraction

Sr.no	Group	Time in days								Epithelialization period
		2	4	8	12	16	18	20	24	
1	Negative control	14.19± 2.907	22.21± 2.738	33.31±4.195	43.30± 3.197	66.81± 2.545	93.45± 1.041	98.13± 2.914	100	22.00± 0.707
2	Standard 0.5%	13.67± 6.186 NS	25.12± 7.215*	51.41±7.112*	75.03± 3.153*	98.84± 1.022*	100*	100NS	100	15.60± 1.517*
3	F3	14.00± 5.283 NS	28.35± 4.745*	51.81±5.165*	76.31± 3.773*	91.55± 1.950*	99.84± 0.25*	100NS	100	16.80± 1.643*
4	F4	18.38± 2.577 NS	31.508± 5.305*	50.40±7.128*	79.01± 4.345*	99.19± 0.592*	100*	100NS	100	14.80± 0.837*

Values are expressed as MEAN±SD at n=6, One-way ANOVA followed by Bonferroni test, *P<0.050 and NSP>0.001 compared to the negative control

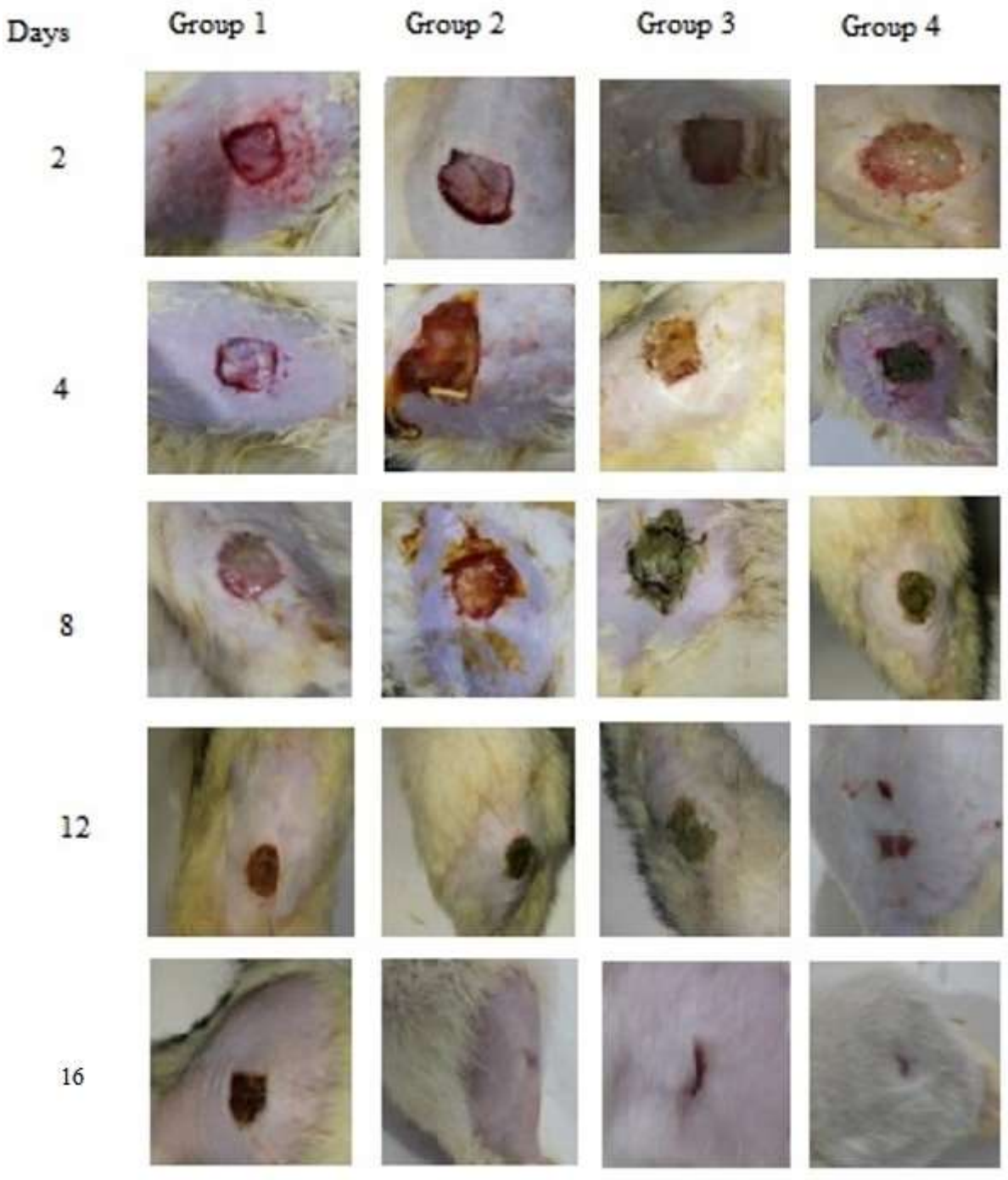

Figure 1: Photographs showing wound repair at different time interval in excision wound model in rats

During the course of study the Polyherbal cream F4 has started to shown their effect from day 4 to day 16 the resultant effect due to complete wound contraction and epithelization shown on day 16 by the standard treated and formulation F4 treated group, While compared with the data of negative control the herbal formulation proved their efficacy in wound healing with significant level of ($P < 0.050$) compared to negative control group.

CONCLUSION

The prepared poly herbal cream was found satisfactory in all aspects of evaluation. The herbal cream has all acceptable qualities like homogeneity, appearance, spredibility, non-greasy, easy removal, pH nearer to skin pH, required viscosity and o/w type emulsion. Formulation F4 was found as effective wound healing agent while compared with the negative control, due to significant reduction in epithelialization period and wound contraction, the formulation F4 could be used as wound healing agent for cure and management of wound. Although wound healing is a complex biological process that involves series of events in complete wound healing, further study needed to find out the specific steps and process involved in wound healing potential of formulation.

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