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Original Article

DEVELOPMENT, OPTIMIZATION AND EVALUATION OF DIFFERENT HERBAL FORMULATIONS FOR WOUND HEALING

FEDELIC ASHISH TOPPO, RAJESH SINGH PAWAR

Pharmacognosy and Phytochemistry Laboratory, VNS Group of Institutions, Bhopal, Madhya Pradesh 462044, India
Email: ashish_two@yahoo.co.in

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ABSTRACT

Objective: This study was done for comparative evaluation of different herbal formulations used for topical delivery of therapeutic agents at the time of injury to accelerate skin repair in the shortest time possible, with minimal pain, discomfort and scarring to the patient during the wound healing process.

Methods: Topical application of ointments and gels prepared from the methanolic extract of *Aegle marmelos* leaves and *Mucuna pruriens* seeds were formulated and evaluated for its efficacy and safety. General formulation approaches towards achieving optimum physical properties and topical delivery characteristics for an active wound healing dosage form were considered for different herbal formulations.

Results: All formulation showed good stability during storage and no major changes observed after carrying out other physiochemical evaluations and during entire storage period. Differences in wound healing were observed between the various treatments and compared to the herbal formulation which has promising effect on the wound healing process.

Conclusions: The results obtained were encouraging and gel-based formulations produced better wound healing than emulsifying ointment formulations. The results demonstrated that the tested hydrogel had promising healing effect in skin injuries and it will aid in identifying and targeting the many aspects of the complex wound healing process.

Keywords: *Aegle marmelos, Mucuna pruriens*, Wound healing, Herbal formulation, Ointment, Hydrogel.

INTRODUCTION

Skin serves as a barrier to water and various pathogens. Wounds and injuries destroy this barrier that normally prevents invasion of bacteria, fungi and viruses[1]. Skin is often injured by wounding or physical trauma, and damages to skin initiate a series of complicated and well orchestrated events of repair processes ending with complete reestablishment of the integrity of damage tissue and restoration of this functional barrier [2-4]. In some severe conditions (i.e. Large full-thickness skin defects), complete re-epithelialization takes a long time [5-8]. Clinicians are still searching for an ideal wound dressing which would provide prompt adherence, water vapour transport, good elasticity and durability. It would create a bacterial barrier, have good antiseptic effects and lack of toxicity and antigenicity. It would have a low cost and markedly reduce the total required treatment cost. Several approaches are currently utilized to treat pain, inflammation, skin diseases, for disinfection of skin and as controlled release devices in the field of wound dressing.

Several drugs obtained from plant sources are known to increase the healing of different types of wounds. Traditionally *Mucuna pruriens* L. (Fabaceae) seeds are applied on cuts and wounds [9, 10] and *Aegle marmelos* L. (Rutaceae) leaves are also used for cut and wound [11, 12]. The last decades bring natural remedies into the medical forefront, having as major role the use of plants in the treatment of different disorders. The concept of phytotherapy treatment is reconsidered by achieving in vivo and in vitro studies regarding the confirmation of the healing effects of plants, the determination of the active principles responsible for these effects, and the elucidation of their mechanism of action [13-15]. In few cases, active chemical constituents were identified [16]. Plants or chemical entities derived from plants need to be identified and formulated for treatment and management of wounds.

By definition, the wound is already a traumatic insult to the body's integrity and any additional trauma (Mechanical or chemical) inflicted in your attempts to manage that wound will only delay the reparative process. In spite of availability of excellent herbal wound healing products and formulations, it has always been difficult and challenging to formulate and deliver the naturally derived drug as an optimum system which can maximize its clinical effect with minimum side effects. Recently many researches have been done for the purpose of solubilization of insoluble or only slightly soluble drug with the application of various nonionic surfactants such as labrasol, tween 80, PEG 400, and so on [17-19]. Also the similar studies have been investigated using anionic surfactants such as Na-taurocholate, Nataurodeoxycholate, Na-deoxycholate, and so on [20, 21, 22]. Especially many delivery systems have been developed to improve the solubilization of drugs by these surfactants.

One of the most promising agents is the system using the interaction of polymer and surfactant. Polymer/surfactant interactions may cause dramatic changes in the drug solubilizing capacity, rheological properties of polymer aqueous dispersions, and in drug diffusion and penetration through the skin and mucouses. In consequence, incorporation of polymer/surfactant opens a wide range of possibilities for developing drug delivery systems [23, 24]. Surface active agents can be segregated according to the charge on the active chemical. They can be designated as cationic when they have a positive charge; anionic when they have a negative; or amphoteric agents which have both positive and negative charges in the same molecule. These charged materials interact with the cell membrane and perturb it. The membrane loses permeability, which ultimately leads to death. So all of these agents are toxic to tissue defenses and inhibit wound healing. If all of the charged particles are going to pose problems, what is left? There is a large chemical group of polymers that are known as non-ionics because they don't carry a charge on them, but even within this group, the majority of non-ionics still have sufficient reactivity to interact with cell membranes and cause problems.

Hence, there is dearth of rational pro-healing agents for the wound management programme, which can hasten the healing process. If patients at risk are identified sooner and aggressive interventions are taken before the wound deteriorates and complications occur,

both patient morbidity and health-care costs can be significantly reduced. The question is: which interventions, technologies and dressing materials are the best from those available. This study attempts to compare and investigate the influence of the presence of various amounts of anionic, cationic and nonionic surfactants which are used in formulating different topical therapeutic herbal formulation. The investigation was concerned with evaluating the effect of different herbal formulation on wound healing intended for topical use in developing countries.

MATERIALS AND METHODS

Materials

All the chemicals used in the study were of analytical grade. The leaves of *Aegle marmelos* (200 gm) and powdered seeds (200 gm) of *Mucuna pruriens* were extracted in soxhlet assembly for 36 hours with petroleum ether for defatting. The defatted plant materials were dried and then exhaustively extracted with methanol in soxhlet apparatus. The completion of extraction was confirmed by evaporating a few drops of the extract on the watch glass and ensuring that no residue remained after evaporating the solvent. The extracts were concentrated under reduced pressure at a bath temperature below 50° C to yield semisolid mass and stored in well-closed container for further studies.

Animals

The Wistar-albino rats (220±20 g) were procured from animal house of VNS group of Institutions, Faculty of Pharmacy, Bhopal MP and maintained under constant conditions (temperature 25±2^{0}C, humidity 40-60%, 12h light/12h dark cycle). During maintenance the animals received a diet of food pellet supplied from animal house and water *ad libitum.* These experiments were approved by the Institutional Animal Ethics Committee, VNSFP, Bhopal MP (VNISP/IAEC/2011/6695/A)

Methods

Preparation of topical herbal formulations

The formulation compositions used were listed in table 1**.** Ointment base was prepared by mixing the ingredients (wool fat 5g, hard paraffin 5g, cetostearyl alcohol 5g, soft white paraffin 85g) as per British Pharmacopoeia (1980) in a beaker at 65^{0}C water bath. After cooling, the mixture was homogenized by a homogenizer at 1500 rpm for 10–15 min [25].

Methanolic extract (s) were respectively added to the melted base at 40^{0}C and stirred continuously until homogenous formulation base is obtained. Three different emulsifying ointments representing anionic, cationic and non-ionic types respectively were prepared employing fusion method. The required quantity of the ointment base was weighed and melted at a temperature of about 70^{0}C in a hot water bath. The designated quantity of the extract (s) was respectively added to the melted base at 40^{0}C and the mix, stirred gently and continuously until a homogenous dispersion is obtained [26]. The gel base formulations was prepared by dispersing one gram of carbopol 934 in 50 ml of distilled water with continuous stirring and kept overnight to get a smooth gel. 2 ml of distilled water was taken and the required quantity of sodium metabisulphite was dissolved by heating on water bath. 5 ml of distilled water was taken and required quantity of methyl paraben and propyl paraben were dissolved by heating on water bath. The solution was then cooled to add sodium metabisulphite solution. Finally, fully mixed ingredients were mixed properly with the carbopol gel with continuous stirring. Then, the required amount of methanolic extract (s) was mixed in the above mixture and its volume was increased to 100 ml by adding distilled water and triethanolamine was added dropwise to the formulation for adjusting the required skin pH (pH: 6.5-7.0) and to obtain required consistency [27] (table 2).

Table 1: Formula of the composition of herbal formulations

Ingredients	Simple ointment base B. P. (SOB)	Anionic emulsifying ointment (AEO)	Cationic emulsifying ointment (CEO)	Non-ionic emulsifying ointment (NEO)	Gel base formulation (Hydrogel) (GBF)
Emulsifying wax BP	_	30%	_	_	_
Liquid paraffin	_	20%	_	_	_
White soft paraffin	85%	50%	_	_	_
Shea butter	_	_	50%	60%	_
Cetostearyl alcohol	5%	_	45%	36%	_
Cetrimide	_	_	5%	_	_
Tween 65 EP	_	_	_	4%	_
Wool Fat	5%	_	_	_	_
Hard Paraffin	5%	_	_	_	_
Carbopol 934	_	_	_	_	2%
Methyl Paraben	_	_	_	_	0.02%
Propyl Paraben	_	_	_	_	0.002%
Sodium metabisulphite	_	_	_	_	0.2%
Triethanolamine	_	_	_	_	QS
Purified water	_	_	_	_	QS to 100 ml

Table 2: Formula of the composition of medicated herbal formulations

Formulations	*Aegle marmelos* Methanolic extract (AMMeoH)	*Mucuna pruriens* Methanolic extract (MPMeoH)
Simple ointment base (F-1)	SOB (97.5%)+AMMeoH (2.5%)	_
Anionic emulsifying ointment (F-2)	AEO (97.5%)+AMMeoH (2.5%)	_
Cationic emulsifying ointment (F-3)	CEO (97.5%)+AMMeoH (2.5%)	_
Non-ionic emulsifying ointment (F-4)	NEO (97.5%)+AMMeoH (2.5%)	_
Gel base formulation (Hydrogel) (F-5)	GBF (97.5%)+AMMeoH (2.5%)	_
Simple ointment base (F-6)	_	SOB (97.5%)+MPMeoH (2.5%)
Anionic emulsifying ointment (F-7)	_	AEO (97.5%)+MPMeoH (2.5%)
Cationic emulsifying ointment (F-8)	_	CEO (97.5%)+MPMeoH (2.5%)
Non-ionic emulsifying ointment (F-9)	_	NEO (97.5%)+MPMeoH (2.5%)
Gel base formulation (Hydrogel) (F-10)	_	GBF (97.5%)+MPMeoH (2.5%)

Evaluation of topical herbal formulations

Appearance and homogeneity

All developed herbal formulations were tested for physical appearance and homogeneity by visual observation. They were tested for their appearance and presence of any aggregates.

pH

1.0 g of herbal formulations were accurately weighed and dispersed in 100 ml purified water. The pH of the dispersion was measured using digital pH meter, which was calibrated before use with the standard buffer solution at 4.0, 7.0 and 9.0. The measurements of pH were done in triplicate and average values were calculated.

Viscosity

The measurement of the viscosity of the prepared herbal formulations was done with Brookfied viscometer (Model RVDVE230). The reading was taken at 100 rpm using spindle no. 7.

Spreadibility

The Spreadibility of the gel formulations was determined by measuring the spreading diameter of 1g of gel between two horizontal plates (20 cmx20 cm). The standard weight applied on the upper plate was 100 g. Spreadibility was determined using the formula (S=MxL/T) where S= spreadibility, M= weight applied, L= length of the glass slides and T= time taken to separate the glass slides completely from each other.

Skin irritation study

The Wistar-albino rats of either sex weighing 220±20 g were used for this test. The hairs were removed from the rat 3 days before the experiment. The herbal formulations containing extracts were used on test animals. Simple ointment base applied on the back of animal was taken as control. The animals were treated daily up to seven days and finally the treated skin was examined visually for erythema and edema [28].

Primary Dermal Irritation Index (PDII) = PDII observed on 12+24+48+72 hrs / 4

Primary dermal irritation index scores for erythema (no erythema-0, very slight erythema-1, well-defined erythema-2, moderate to severe erythema-3, severe erythema-4) and edema (no edema-0, very slight edema-1, well-defined edema-2, moderate to severe edema-3, severe edema-4)

Evaluation of wound healing activity

The animals were divided into 11 groups of 6 rats each. Group I was served as control treated with simple ointment base, group II to XI were served as test groups treated with herbal formulations F-1 to F-10 respectively. Dorsal hairs at the back of the rats were removed by hair remover cream. Rats were anaesthetized by anesthetic ether prior to excision. A circular wound of about 1 cm was made on depilated dorsal thoracic region of rats under aseptic conditions and was observed throughout the study. Wound index was measured daily with an arbitrary scoring system [29] (Table-5&6).

Table 3: Evaluation parameters of prepared topical herbal formulations

Formulations	Appearance	Homogeneity	pH	Spreading diameter after 1 min (mm)	Viscosity (cp)
Ointment base	White	Good	7.1±0.08	42	6480
F-1	Green	Good	6.9±0.05	49	6100
F-2	Green	Good	6.8±0.03	50	5840
F-3	Green	Good	6.7±0.03	50	5880
F-4	Green	Good	6.5±0.03	52	5380
F-5	Green	Good	6.6±0.03	69	5120
F-6	Brown	Good	6.8±0.05	48	6400
F-7	Brown	Good	6.8±0.06	49	5933
F-8	Brown	Good	6.8±0.03	49	5902
F-9	Brown	Good	6.6±0.05	50	5890
F-10	Brown	Good	6.5±0.03	64	5200

Table 4: Skin irritation study of prepared topical herbal formulations

Experimental groups	Reactions	Primary Dermal Irritation Index (PDII) Score PDII observed on 12+24+48+72 hrs / 4
Control group I	Erythema	0.83
(Ointment base)	Edema	0.29
Group II	Erythema	0.5
(F-1)	Edema	0.12
Group III	Erythema	0.25
(F-2)	Edema	0.12
Group IV	Erythema	0.20
(F-3)	Edema	0.04
Group V	Erythema	0.08
(F-4)	Edema	0
Group VI	Erythema	0.04
(F-5)	Edema	0
Group VII	Erythema	0.41
(F-6)	Edema	0.08
Group VIII	Erythema	0.25
(F-7)	Edema	0.12
Group IX	Erythema	0.16
(F-8)	Edema	0.08
Group X	Erythema	0.08
(F-9)	Edema	0
Group XI	Erythema	0.04
(F-10)	Edema	0

<0.5: non-irritating, 0.5-2.0: slightly irritating, 2.1-5.0: moderately irritating and>5.0: severely irritating.

Table 5: Arbitrary scoring system for the measurement of wound index

Gross changes	Wound index
Complete healing of wounds	0
Incomplete but healthy healing	1
Delayed but healthy healing	2
Healing has not yet been started but the environment is healthy	3
Formation of pus-evidence of necrosis	4

Table 6: Effect of different prepared topical herbal formulations on wound healing in different treatment groups

Experimental groups	Days required for complete healing	Wound index
Control group I	12.5±1.25	1.74±0.02
Group II	8.0±1.05*	1.31±0.03**
Group III	7.5±0.98**	1.22±0.06**
Group IV	7.5±0.98**	1.18±0.04**
Group V	7.5±0.98**	1.15±0.02**
Group VI	7.5±0.98**	1.07±0.02**
Group VII	8.0±1.05*	1.33±0.03**
Group VIII	7.5±0.98**	1.17±0.04**
Group IX	7.5±0.98**	1.16±0.03**
Group X	7.5±0.98**	1.15±0.02**
Group XI	7.0±0.91**	1.07±0.02**

Number of animals (n=6), Values are mean±S. E. M., *P<0.05, **P<0.01. P value was calculated by comparing control *vs* treatment groups by Dunnett's t-test using the software Graph Pad Instat.

Statistical analysis

The experimental results were expressed as the mean±S. E. M. (standard error of mean) and the statistical significance was evaluated by one way analysis of variance (ANOVA) followed by Dunnett's t-test using the software Graph Pad Instat.

RESULTS AND DISCUSSION

The physicochemical properties of the simple ointment base and topical herbal formulations were shown in table 3. From the results it is evident that all topical herbal formulations and ointment base showed uniform homogeneity and spreadability. The physical appearances of the simple ointment base was white and topical herbal formulations of *Aegle marmelos* and *Mucuna pruriens* methanolic extracts were found to be green and brown in colour respectively. The pH of the simple ointment base and topical herbal formulations were in the range of 6.5±0.03 to 7.1±0.08, which lies in the normal pH range of the skin and would not produce any skin irritation. There was no significant change in pH values as a function of time for all formulations.

This also indicated that the selected ingredients of the formulation did not alter the pH of the formulation. The values of spreadability indicated that the simple ointment base and topical herbal formulations were easily spreadable by small amount of shear. Spreading diameter after 1 min (mm) of simple ointment base and topical herbal formulations were between 42-69 mm which indicates good spreadability of simple ointment base and topical herbal formulations. The results indicated that the formulation can be applied easily without being runoff. This assures that the formulation maintain a good wet contact time when applied to the site of application. Viscosity is the most important parameter in the evaluation as it governs the many properties of the formulation such as, spreadability, pourability of the product from the container etc. The viscosity of the gel formulations generally reflects its consistency. Viscosity of the simple ointment base and topical herbal formulations were determined by using Brookfied viscometer and were ranging between 5120 to 6480 centipose.

The results of skin irritancy studies indicated that the simple ointment base and topical herbal formulations were free from dermatological reactions. All the formulations did not produce any skin irritation, i.e., erythema and edema for about 72 hrs when applied over the skin (Table 4). In wound healing process cellular structures and tissue layers in damaged tissue are restored as closely as possible to its normal state. In wound healing study, healing progressed in wound treated with simple ointment base and topical herbal formulations. Regarding the mean number of days of complete healing, the least number was required in group XI (P<0.01) and in group II and group VII were significantly high (P<0.05) with respect to the value of control group. The mean wound index of each animal of different treatment groups were measured daily. All the results were found significant (P<0.01) and healthy healing were observed in all the treated groups with simple ointment base and topical herbal formulations. Mean wound index of gel formulations were found significantly decreased as compared with control group. (table 6).

Ointment bases are almost always anhydrous and generally contain one or more medicaments in suspension or solution or dispersion. Ointment bases may be hydrocarbon (oligeanous), absorption, water removable and water soluble type. On the basis of their level of action, they are classified as: epidermatic, endodermatic and diadermatic [30]. The hydrophilicity and lipophilicity of drug influence the drug permeability through skin. Lipophilic drugs penetrate faster through skin especially when the carrier system is lipophilic as well [31]. For lipophilic drugs, lipid concentration added in the gel has a strong affect on the drug release and the rigidity of membranes is not important [32].

Among the currently used polymers, the hydrogel has been widely used for preclinical and clinical studies. In the preparation of hydrogel typed ointment, Carbopol has been widely used both in pharmaceutical and cosmetic preparations [33]. Carbopol resins are cross-linked polyacrylic acids. They are hydrated in the presence of water, and the carboxylic acid groups in the molecules can dissociate in an aqueous system. The negative charges on the polymer backbone repel each other to cause dramatic polymer expansion. Its swelling and hydrophilic characteristics allow three main pharmaceutical applications; (i) as a thickening agent [34], (ii) as a suspending agent [35], and (iii) as an emulsifying agent [36]. Wound dehydration disturbed the ideal environment to stimulate wound healing process; therefore, maintenance of a moist wound bed is of great importance for effective wound healing. Hydrogel could absorb tissue exudates, prevent wound dehydration, and allow oxygen to permeate [37].

The prepared gel formulations had promising effect on the wound healing process. The physicochemical properties of prepared gel formulations were in good agreement. Among all the formulations applied on damaged skin, hydrogels have shown the superiority as

they can provide a moist environment for the wound and at the same time deliver the incorporated drug to the wound.

CONCLUSION

The greater part of the world's population relies on traditional medicine for their health care. This is also the case in the treatment of wounds. In developing countries, formulations prepared from plants have been widely used for the treatment of soft tissue wounds and burns by medical personnel trained in western medicine as well as by traditional practitioners. In present work, attempt was made to develop, optimize and evaluate different herbal formulations for wound healing. The ultimate aim was comparative evaluation of different herbal formulations used for topical delivery of therapeutic agents at the time of injury to accelerate skin repair in the shortest time possible, with minimal pain, discomfort and scarring to the patient during the wound healing process. The mechanical evaluation parameters like pH, viscosity, spreadability, homogeneity are important tests to evaluate pharmaceutical topical formulations.

Development of an ideal wound healing herbal drug is still a challenge to the medical scientists. The ideal drug should fulfill the criteria such as healthy healing, rapid contraction of wound leading to quick healing, reduction of wound index etc. It is inferred from results that the gel formulations as compared to other formulations were found to be good in appearance, homogeneity and easily spreadable and showed significant lowering of wound index and number of days required for complete healing in Wistar rat models.

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CONFLICT OF INTERESTS

Declared None

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