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Research Article

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Formulation and Evaluation of Neomycin Sulphate Ointment containing Natural Wound Healing Agent *Curcuma longa*

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ABSTRACT
Present investigation was to develop novel ointment formulation in combination of natural wound healing agent *curcuma longa*, which is reported to possess wound healing and anti-bacterial activities. Combination of neomycin sulphate and *curcuma longa* is good rational, where *curcuma longa* produces synergistic wound healing effect with neomycin sulphate. Formulations containing fixed concentration (0.5 %) of neomycin sulphate and 3 %, 4 % and 5 % of *curcuma longa* were prepared. To assess the efficacy of formulations anti-bacterial activity, rheology, stability, spreadability and other physical characteristics were evaluated. The results obtained were encouraging and formulation containing neomycin sulphate (0.5 %) with 5 % of *curcuma longa* was found better than other formulations.

Keywords: Ointment; Neomycin sulphate; *Curcuma longa;* wound healing.

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INTRODUCTION

A wound has been defined as loss or breaking of cellular and anatomic or functional continuity of living tissue. The vast literature on wound healing is mainly focused on skin, which is the most susceptible organ in the body that interacts with the environment and therefore receives constant insult and damage.

Wound healing 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 are later remodeled to form scar.

Wound healing involves a complex interaction between epidermal and dermal cells, the extra cellular matrix, controlled angiogenesis and plasma-derived proteins all coordinated by an array of cytokines and growth factors. This dynamic process is classically divided into three overlapping phases – "inflammation, proliferation and remodeling". [1] There are various natural agents, which assist in wound healing process. This treatment provides fibrogenetic and concentration of collagen resulting in faster healing.

The drug selected for this work was Neomycin sulphate [2], which is an aminoglycoside antibiotic that works by binding to the bacterial 30S ribosomal subunit, causing misreading of t-RNA, leaving the bacterium unable to synthesize proteins vital to its growth. Neomycin sulphate was selected because it was easily available and its estimation was possible with reasonable accuracy in the conditions prevailing in the laboratory. *Curcuma longa* (powder) was used in combination with neomycin sulphate. *Curcuma longa* [3] is reported to have anti-bacterial [4] and anti-inflammatory activities, which are complementary to wound healing process. The easy availability of *curcuma longa*, cost-effectiveness and reduction of microbial resistance against neomycin sulphate, prompted us to formulate topical neomycin sulphate ointment in combination with *curcuma longa*. The combination was used to enhance the wound healing activity.

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MATERIALS AND METHODS

Materials

Neomycin sulphate was received as a gift sample from Modern laboratories, Indore, India. Emulsifying wax, white soft paraffin, liquid paraffin and all other chemicals were of analytical grade and used without further purification. Bacterial culture of *Staphylococcus aureus* and *Escherichia coli* were obtained from Department of Biotechnology, IPS Academy, Indore, India.

Method for preparation of ointment

Emulsifying wax, white soft paraffin and liquid paraffin were heated to 70-75°C to melt it completely. Then neomycin sulphate and / or *curcuma longa* was / were dissolved in it

under stirring and then cooled. The composition [5] of emulsifying ointment base is given in Table 1 and composition of different ointment formulations is given in Table 2.

Table 1: Composition of emulsifying ointment base

Ingredients	Quantity (%)
Emulsifying wax	30
White soft paraffin	50
Liquid paraffin	20

Table 2: Composition of different ointment formulations

Item	Material name	Quantity (%)						
		F1	F2	F3	F4	F5	F6	F7
1.	Neomycin sulphate	0.5	0.5	0.5	0.5	--	-	--
2.	*Curcuma longa*	----	3	4	5	3	4	5
3.	Emulsifying ointment base	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.

Table 3: Inhibition zone diameters of different formulations

Formulations	ZoI for *S. aureus* (mm *)	ZoI for *E. coli* (mm *)
F-1	22.3	22.9
F-2	24.0	24.2
F-3	28.5	30.4
F-4	31.3	35.8
F-5	15.2	16.6
F-6	17.7	18.1
F-7	18.4	19.0

* ZoI- Zone of inhibition. Values are average of three determinations.

Anti-bacterial activity

The antibacterial activity of various ointment formulations of neomycin sulphate and *curcuma longa* against *Staphylococcus aureus* and *E. coli* was evaluated by the standard cup plate method [6] and the inhibition zone diameters were measured (Table 3). Nutrient agar media was used for bacterial culture and incubated at temperature of 37°C ± 2°C for 24 h.

Spreadability

Spreadability was determined by modified wooden block and glass slide apparatus. The apparatus consisted of a wooden block with fixed glass slide and a pulley. A pan was attached to another glass slide (movable) with the help of a string. For the determination of spreadability measured amount of ointment was placed in the fixed glass slide, the movable glass slide with a pan attached to it, was placed over the fixed glass slide, such that the ointment was sandwiched between the two slides for 5 min. The weight was continuously removed. Now about 50 g of weight was added to the pan. [7] Time taken for the slides to separate was noted. Spreadability was determined using the following formula:

$$S = M/T$$

Where *S* is the spreadability in g/s, *M* is the mass in grams and *T* is the time in seconds.

Extrudability

A closed collapsible tube containing ointment was pressed firmly at the crimped end. When the cap was removed, ointment extruded until pressure dissipated. Weight in grams required to extrude 0.5 cm ribbon of ointment in 10 seconds was determined. [8]

Viscosity

Brookfield digital viscometer (model DV-I+, Brookfield Engineering Laboratory, INC., USA) was used to measure the viscosity (in cps) of the prepared ointment formulations as such that is in semisolid state. The spindle T-D (spindle code S 94) was rotated at 2.5, 4, 5 and 10 rpm. The reading, near to 100% torque was noted. Samples were measured at 30 ± 1° C.

Skin irritation study

Three albino rabbits were selected for the study. 24 h prior to the test, the test sites were depilated on both sides of the spine and demarcated for the application of the formulation. The measured quantity of ointment was applied over the respective test sites. The test sites were observed for the erythema and edema for 48 h after application.

Stability of formulations

Ointment formulations were evaluated in terms of physical changes like phase separation and changes in color, odour, consistency etc., thereby affecting their stability and other desired properties. Samples of the ointment formulations were kept at different temperature conditions like 25°C, 30°C and 40°C for 45 days. They were periodically observed for physical changes like phase separation and development of objectionable color and odour etc. Anti-bacterial activities of formulations were also estimated after 45 days. [9]

RESULTS AND DISCUSSION

The aim of the present work was to verify the hypothesis that *curcuma longa* by providing better tissue formation and neomycin by providing protection against microbial invasion would prove a superior treatment for healing wounds if given concomitantly preferably in one formulation. Somewhat consequent to this objective it became necessary to develop a formulation containing these two active medicaments namely *curcuma longa* and neomycin. It became consequential to examine whether such a formulation satisfies the normal criteria for a formulation to be accepted as a medicinal agent. The present work aims to evaluate whether combination of *curcuma longa* and antibiotic produces any synergistic effect on wound healing. *Curcuma longa* has been traditionally used as a wound healer in ancient days. As a result of the discovery of newer antibiotics [10] and related chemotherapeutic agents, use of *curcuma longa* went into total obscurity. Ability of microorganisms to render themselves resistant to antibiotics necessitated search for newer and newer agents. [11] During such efforts it was noticed that *curcuma longa* promotes wound healing [12] even without any application of antibiotics and its activity is almost parallel to antibiotic. This observation leads to logical reasoning that combination of *curcuma longa* and an antibiotic may provide umbrella during the process of healing. It is therefore logical to attempt a formulation containing c*urcuma longa* and antibiotic and evaluate it with the usual parameters. Consequently the present work aimed at preparation and evaluation of such formulation. Ointment was the obvious choice of dosage form because that is the most convenient form of topical application. Conjoint administration of a suitable antibiotic may enhance the efficacy although application of *curcuma longa* per se is also effective.

The formulations were evaluated for anti-bacterial activity, spreadability, viscosity, extrudability, skin irritation and stability. From the results, it is clearly evident that all formulations showed good extrudability, viscosity and spreadability. The result of spreadability varies from 4.95 to 6.47g/sec. and shown in Table 4 where as the extrudability of ointment formulations from the collapsible tube, varies from 180 to 190 g and shown in Table 4. The viscosity of formulations ranges from 14410 cps to 15213 cps at 10 rpm and shown in Table 4. No signs of erythema and edema were found after 48 h of application in albino rabbits. The anti-

Table 4: Stability evaluation data of Neomycin sulphate ointment containing natural wound healing agent Curcuma longa

Formulations	Spreadability (g./sec)	Extrudability (g)	Viscosity (cps) at rpm			
			2.5	4	5	10
F-1	4.95	180	65187	34470	25180	14410
F-2	5.53	180	65562	34846	26123	14735
F-3	6.12	185	65896	35141	26547	14982
F-4	6.47	190	66180	35583	26996	15213
F-5	5.26	180	65235	34697	25953	14648
F-6	4.79	185	65802	34984	26355	14827
F-7	5.28	185	66094	35108	26870	15174

bacterial activity of different formulations was determined initially and after stability period. No significant difference was found in anti-bacterial activity of different formulations after 45 days of stability period. From the data, it is evident that formulation F-4 containing both neomycin sulphate (0.5 %) and *curcuma longa* (5 %) showed larger zone of inhibition in comparison to other formulations. Hence in wound healing activity formulation F-4 found to be superior to other formulations. Results of all other evaluation parameters of F-4 were also satisfactory among all the formulations. It can be concluded that *curcuma longa* produces significant synergism with neomycin sulphate for wound healing. The formulation was physically and chemically stable for at least 45 days at 40°C.

From all the above studies, the formulation F-4 containing *curcuma longa* (5 %) + neomycin sulphate (0.5 %) was quite stable and found to be superior to other formulations.

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