IJP (2018), Vol. 5, Issue 8 **(Research Article)**

Received on 28 April, 2018; received in revised form, 08 June, 2018; accepted, 13 June, 2018; published 01 August, 2018

POLY HERBAL SEMISOLID DOSAGE FORM DEVELOPMENT FOR WOUND HEALING

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Keywords:

Terminalia chebula, Cucimum cyminum, Trachyspermum ammi, Piper nigrum, Zingiber officinalis

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ABSTRACT: Purpose: The aim of the present study was to investigate the wound healing activity of the selected Indian medicinal plants *Terminalia chebula, Terminalia bellarica, Ficus racemosa, Piper nigrum, Zingiber officinalis.* **Method:** Excision and incision models for diabetes induced rats at specified varying doses. **Result:** The plants showed a definite, positive effect on wound healing. **Conclusion:** The efficacy of this plants in wound healing may be due to its action on antioxidant enzymes, thereby justifying the traditional claim.

INTRODUCTION: Wound healing or wound repair, is an intricate process in which the skin (or another organ) repair itself after injury. In normal skin, the epidermis (out outer most layers) and dermis (the inner most layer) exists in steady state equilibrium, forming a protective barrier against the external environment. Once the protective barrier is broken, the normal (physiologic) process of wound healing is immediately set in motion. *Piper longum* and *Terminalia chebula* plants were found to offer protection against these stressors and black piper (*Piper nigrum*) were investigated for their antioxidant and radical scavenging activities, *Terminalia bellerica* and finally identified as the compound was having hepato-protective activity and the management of wounds.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.IJP.5(8).461-66

Article can be accessed online on: www.ijpjournal.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.5(8).461-66

Triphala (dried fruits of *Terminalia chebula*, *Terminalia bellarica* and *Phyllanthus emblica*) and the treated group has shown significantly improved wound closure. Atsushi Kato *et al*., (2006) investigated on Ginger (*Zingiber officinale* Roscoe) continues to be used as an important cooking spice and herbal medicine around the world.

Scientific research has gradually verified the antidiabetic effects of ginger [1, 2, 3, 4]. Based on the earlier claims the following plants were selected for the proposed study diabetic wound healing activity plants- *Terminalia chebula* (Combretaceae), *Terminalia bellerica* (Combretaceae), *Ficus racemosa* (moraceae), *Piper nigrum* (Piperaceae), *Zingiber officinalis* (Zingiberaceae).

MATERIALS AND METHODS:

Plant Collection: *Terminalia bellerica* (fruit)**,** *Terminalia chebula* (fruit), *Cuminum cyminum* (seed), *Piper nigrum* (fruit), *Zingiber officinalis* (rhizome) and are collected from authorized suppliers.

Poly Herbal Formulation: The collected materials are dried in sunlight for few days and grinded all together to make into powder form. The polyherbal powder was mixed with simple ointment in a porcelain tile and transferred to a tightly-closed amber colored container. For topical administration, PHF 5% w/w was prepared by adding 5 gm of polyherbal powder to the 100 gm of simple ointment base I.P. and PHF 10% w/w ointment was prepared by adding 10 gm of polyherbal powder in 100 gm of simple ointment base i.p, packed in an airtight container and stored at room temperature.

TABLE 1: COMPOSITION OF POLYHERBAL POWDER

S. no.	Ingredient	Parts of plant used	Quantity for 1000 gm
1	*Terminalia bellerica*	Fruit	100gm
2	*Terminalia chebula*	Fruit	100gm
3	*Cuminum cyminum*	Seed	100gm
	Piper nigrum	Fruit	100gm
5	*Zingeber officinalis*	Rhizome	100gm

TABLE 2: COMPOSITION FOR SIMPLE OINTMENT

S. no.	Ingredients	for 100gm of simple ointment base	PHF 5%w/w	PHF 10%w/w
1	Wool fat	5gm	5gm	5gm
2	Hard paraffin	5gm	5gm	5gm
3	Yellow soft paraffin	85gm	85gm	85gm
4	Cetostearyl alcohol	5gm	5gm	5gm
5	Polyherbal powder	-	5gm	10gm

Selection and Procurement of Animals: After taking permission for animal studies from Institutional Animals Ethics Committee (IAEC) healthy Albino Rats (XII/VELS/PCOL/25/2000/ CPCSEA/IAEC/08.08.12) were procured. The rats of both sex weighing 150 - 200 gm and 1.5 - 2 kg of Rabbits were selected for the present study. They kept in plastic cages and maintained at 24 - 28 ºC. All the animals are housed individually with free access to food and water *ad libitum*. They are fed with standard diet and kept in well ventilated animal house. They also maintained with alternate dark-light cycle of 12 h throughout the studies. The animals were closely observed for any infection and if they showed signs of infection they are separated or excluded from the study and replaced. The animal experiment is performed accordance with legislation on welfare.

Evaluation of Wound Healing Activity: [5, 6] For assessment of wound healing activity, excision and incision diabetic wound models were used.

Excision Wound Model with Diabetes*:* Overnight fasted animals received freshly prepared alloxan (Sigma Co. St Louis, USA) in normal saline intraperitoneally as a single dose of 120 mg/kg body weight. Forty-eighty hours after alloxan administration, blood samples were obtained via tail bleeding and blood glucose levels were determined to confirm diabetes. The blood glucose level of all the rats checked for diabetic condition was determined. After confirming diabetes, the rats were depilated on the back and cutaneous circular wound of 8 mm diameter were inflicted on the pre-shaved sterile dorsal surface of the animal by cutting in each group each animal received four wound. Animals were housed individually in metallic cages. The wound was left undressed to the open environment. Then the treatment was started in the following pattern:

Group I: control (Simple ointment only).
Group II: Standard (Povidone iodine 5% w/w ointment).
Group III: PHF 5% w/w.
Group IV: PHF 10% w/w.

Drug was applied once a day after cleaning with surgical cotton.

Parameters Used to Assess Wound Healing Activity: [7, 8] To assess the area of the healing wound the surface area was measured by tracing the boundary on semitransparent paper and calculation was done using a graph paper. The percentage of wound closure was recorded on day 0, 5, 10 and 14. Wound area are traced and measured planimetrically with the help of sq. mm graph paper. Number of days required for falling of the scar without any residual raw wound gave the period of epithelization. Histopathological studies of wound tissues stained with erosion and hematoxylin were studied for anglogenesis, fibrogenesis, and epithelization.

Incision Wound Model with Diabetes*:* Similar as above, animals in each group diabetes was induced and then the rats were anaesthetized and one paravertebral long incisions were made through the skin and cutaneous muscle at a distance of about 1.5 cm from the midline on each side of the depilated back of the rat. Full aseptic measures were not taken and no local or systemic antimicrobials were used throughout the experiment. All the groups were treated in the same manner as mentioned in the case of excision wound model. No ligature was used for stitching.

RESULT AND DISCUSSION:

Antimicrobial Property: Antimicrobial properties are useful tools in the control of microorganisms especially in the treatment of infections and food spoilage. PHF were screened for their *in-vitro* inhibitory effects against certain strains of fungi and bacteria. Many plants contain microbial inhibitors.

MIC values obtained for the tested microorganisms are reported in **Table 3** and **Fig. 1**. The PHF produced significant activity against *Candida albicans, Pseudomonas aeroginosa, Salmonella paratyphi* and *Aspergillus niger.*

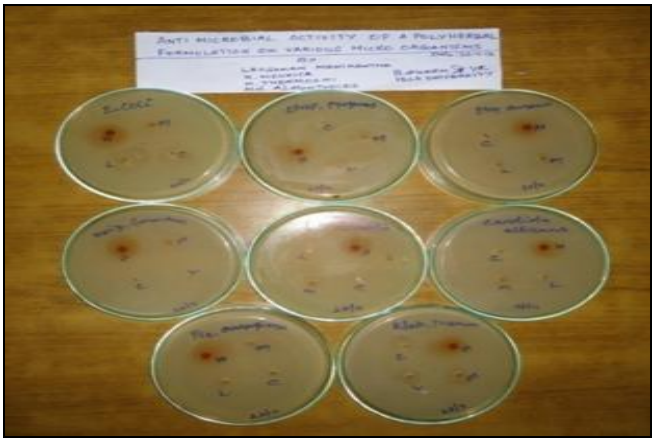

FIG. 1: ANTIMICROBIAL ACTIVITY OF POLY-HERBAL FORMULATION ON VARIOUS MICRO ORGANISMS

TABLE 3: ANTIMICROBIAL ACTIVITY OF PHF

Antimicrobial Agent	Inhibition zones in diameter (mm)				
Concentration	***Escherichia coli***	***Pseudomonas aeroginosa***	***Salmonella paratyphi***	***Candida albicans***	***Aspergillus niger***
Control	07	10	09	07	09
Test 1-25 µg/ml	09	08	05	04	04
Test 2-50 µg/ml	12	12	10	08	09
Test 3-75 µg/ml	03	07	05	06	07

Poly herbal formulation Showed maximum inhibition for the species *Escherichia coli* (12 mm), *Pseudomonas aeroginosa* (12 mm), *Candida albicans* (8 mm), *Salmonella paratyphi* (10 mm) and *Asperigillus niger* (9 mm) at the concentration of 50 µg/ml.

Wound Healing Property: A wound which is disrupted state of tissue caused by physical, chemical, microbial or immunological insult ultimately heals either by regeneration or fibroplasias. Wound healing is the process of repair that follows injury to the skin and other soft tissues. Wounds are still a major problem in developing countries, often having severe complications and involving high costs for therapy.

An important aspect of the use of traditional medicinal remedies and plants in the treatment of burns and wounds is potential to improve healing and the same time to reduce the financial burden. Several plants and herbs have been used experimentally to treat skin disorders, including wound injuries, in traditional medicine. Collagen is a major protein of the extracellular matrix and is the component that ultimately contributes to wound strength [9, 10].

In excision wound model, on days 5 and 10 the wound area of standard and test ointment treated groups was found to be significant ($p < 0.05$) in comparison to control group diabetic animals treated with simple ointment base.

The activity is maximum on the day 14 and there was statistically significant difference in the wound area relative in diabetic animals treated with PHF 5% w/w and PHF 10% w/w showed 52.59% and 52.33% healing respectively compared to standard drug group **Table 4 Fig. 2, 3, 4, 6**. It was also observed that epithelialization periods of PHF 5% w/w and PHF 10% w/w group were shorter in comparison to control group.

On the day 14, the study of the histological structure showed excellent tissue regeneration in the skin wound treated with PHF 5% w/w and PHF 10% w/w and comparable with povidone ointment treated standard group.

TABLE 4: EFFECT OF PHF ON EXCISED WOUND IN DIABETIC RATS

Treatment	Wound contraction (%)				
	Day 1	Day 5	Day 10	Day 14	Epithelialization (day)
Control	1.87±0.5	1.47±0.7 (21.39%)	1.49±0.2 (24.06%)	1.3±0.5 (30.04%)	28.12±0.84
Standard	1.9±0.8	0.85±0.2 (55.26%)	0.67±0.2 (64.73%)	0.25±0.3 (86.84%)	14.11±0.67**
PHF 5% w/w	2.32±0.5	1.8±0.4 (22.41%)	1.72±0.6 (25.86%)	1.22±0.5 (47.41%)	18.20±0.74a**
PHF 10% w/w	1.72±0.7	1.27±1.1 (26.16%)	1.12±0.6 (34.18%)	0.9±0.8 (47.67%)	21.25±0.66a**

Values are mean ± SEM (n=6); **p < 0.05; aP < 0.01 (Comparison made between wounded control and standard).
In excision wound model the wound contraction of PHF 5% w/w and PHF 10% w/w is effective when compare to standard (aP<0.01) and control (**P<0.05).

FIG. 2: EXCISION WOUND MODEL - DAY 1-14

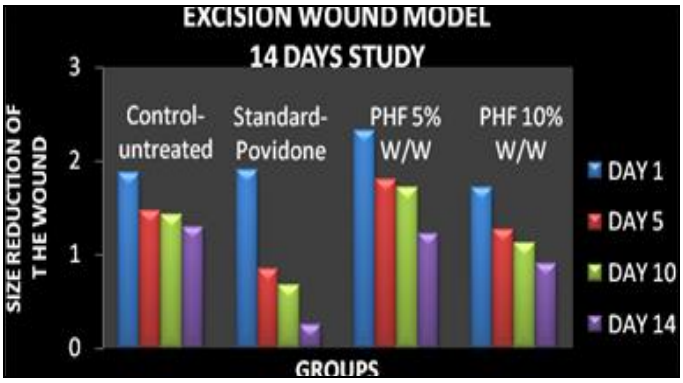

FIG. 3: GRAPH OF EXCISION WOUND MODEL SHOWING REDUCTION IN SIZE OF THE WOUND

FIG. 4: INCISION WOUND MODEL - DAY 1-14

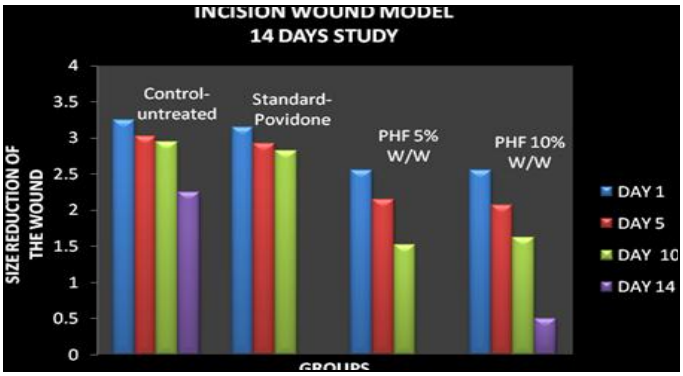

FIG. 5: GRAPH OF INCISION WOUND MODEL IN DAY 14

On the day 14, excision and incision type of wounds in groups of PHF 5% w/w shown 100% healing and PHF 10% w/w shown 80.39% wound healing in diabetic rats as in collagenation, fibroblasts cells, whereas the skin wound treated with simple ointment base presented edema with cellular necrosis that were not observed in the test drug and standard drug treated group of diabetic animals. Wound healing is a process by which damaged tissue is restored as closely as possible to its normal state and wound contraction is the process of shrinkage of the area of the wound **Table 5, 6, 7**.

TABLE 5: EFFECT OF PHF ON INCISION WOUND MODEL IN DIABETIC RATS

Treatment	Breaking Strength (g)	Hydroxyproline mg/g
Control	212±10.82	12.18±2.89NS
Standard	376±12.37**	23.66±4.10NS
PHF 5% w/w	285±10.96a**	17.10±3.00NS
PHF 10% w/w	321±12.20a**	19.64±3.12NS

Values are mean ± SEM for groups of six animals each.
(**P<0.05; aP<0.01) *vs* control and standard, (NSP>0.01) not significant to standard. In incision wound model the wound contraction of PHF 5% w/w and PHF 10% w/w is effective when compare to control (**P<0.05) and standard (aP<0.01) but the hydroxyproline mg/g is Non significant (NS) when compare to standard and control.

TABLE 6: EFFECT OF PHF ON COLLAGEN CONTENT OF THE GRANULOMA TISSUE IN DIABETIC RATS (INCISION WOUND MODEL)

Day	Collagen in mg/g				
	Negative control	Positive control	Standard	PHF 5% w/w	PHF 10% w/w
5	1.23±0.17	0.14±0.05**	0.34 ±0.07**	0.27±0.04*	0.29±0.05**
10	1.25±0.12	0.17±0.04**	0.67±0.06**	0.48±0.05*	0.55±0.06**
14	1.23±0.02	0.19±0.06**	0.99±0.09**	0.75±0.08**	0.80±0.05**

Values are represented as Mean ± SEM for groups of six animals each. (**P<0.01; *P<0.05) *vs.* control. In incision wound model collagen content of PHF 10% w/w is significant to standard (**P<0.01) from the day 5 onwards but the collagen content of PHF 5% w/w is significant to standard only (**P<0.01) from DAY 14.

TABLE 7: PERCENTAGE WOUND CLOSURE OF INCISION WOUNDED RATS

Group	Treatment	Wound closure (%)			
		Day 1	Day 5	Day 10	Day 14
I	Control-untreated	3.25±0.50	3.02±0.5 (7.07%)	2.95±0.05 (9.23%)	2.25±0.50 (30.76%)
II	Standard-povidone	3.15±0.30	2.92±0.65 (7.30%)	2.82 ±0.05 (10.47%)	(100%)
III	PHF 5% w/w	2.55±0.10	2.15±0.30 (15.68%)	1.52±0.05[a**] (40.39%)	(100%)
IV	PHF 10% w/w	2.55±0.10	2.07±0.45 (18.82%)	1.62±0.05[a**] (36.47%)	0.5(80.39%)

Values are as mean ± S.E.M. for groups of six animals each. (**P<0.05) *vs.* Control; ([a]P<0.01) *vs.* standard. In incision wound model the percentage of wound closure in PHF 5% w/w is 40.39% and comparable with standard ([a]P<0.01) and control (**P<0.05) and the percentage of wound closure in PHF 10% w/w is 36.47% and comparable with standard ([a]P<0.01) and control (**P<0.05)

It is mainly dependent upon the type and extent of damage, the general state of health and the ability of the tissue to repair. The main objectives in these processes are to regenerate and reconstruct the disrupted anatomical continuity and functional status of the skin. In the maturational phase, the final phase of wound healing, the wound undergoes contraction, resulting in a smaller amount of apparent scar tissue. Granulation tissue formed in the final part of the proliferative phase is primarily composed of fibroblasts, collagen, edema, and new small blood vessels. In the present study, the wound healing potential in diabetic animals for and PHF 5% w/w and was evident on the day 5 onwards, this potential was further confirmed in the histological evaluation on day 14. No remarkable healing effect was observed with in control group diabetic rats.

On days 0, 5, 10 and 14 animals treated with the PHF 5% w/w showed results similar to animals treated with povidone HCl, with improving the wound healing process. The results in this study are in support that the wound healing and repair is accelerated by applying PHF 5% w/w and PHF 10% w/w, which was highlighted by the full thickness coverage of the wound area by an organized epidermis in the presence of mature scar tissue in the dermis.

CONCLUSION: The present study indicates the wound healing activity of PHF in experimental animal using incision, excision wound models. Wound contracture is a process that occurs throughout the healing process, commencing in the fibroblastic stage whereby the area of the wound undergoes shrinkage. Collagen, the major component which strengthens and supports extracellular tissue, is composed of the amino acid, hydroxyproline, which has been used as a biochemical marker for tissue collagen. Histologic study also substantiates the results and indicates that test drugs stimulates and enhances the faster lay down of collagen fibers with the PHF received diabetic animals than the untreated diabetic control wound.

Hence, based on the results it can be concluded that the test drug used in this study in different concentrations were effective and showing highly beneficial healing responses in the diabetic condition.

ACKNOWLEDGEMENT: We are greatly thankful to the School of pharmaceutical sciences, Vels University and Malla reddy college pharmacy for providing platform to complete the research.

CONFLICT OF INTEREST: We declare that we have no conflict of interest.

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How to cite this article:
Nirmala S, Sudhakar M, Anusri P and Nadanasabapathi P: Poly herbal semisolid dosage form development for wound healing. Int J Pharmacognosy 2018; 5(8): 461-66. doi link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.5(8).461-66.