



AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: <http://www.ajptr.com/>

Preparation and *In-Vitro* Evaluation of Polyherbal Formulation for Wound Healing

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ABSTRACT

Wounds are the result of injuries to the skin that disrupt the other soft tissue. Healing of a wound is a complex and protracted process of tissue repair and remodeling in response to injury. Various plant products have been used in treatment of wounds over the years. Wound healing herbal extracts promote blood clotting, fight infection, and accelerate the healing of wounds. Phytoconstituents derived from plants and identified and screened for antimicrobial activity, and the ratio was selected for the preparation of ointment. The in vitro assays are useful, quick, and relatively inexpensive. The wound healing efficacies of various herbal extracts have been evaluated in CAM assay in in-vitro model. CAM was used to assess the angiogenic activity of polyherbal formulation. Angiogenesis plays an important role in wound healing and newly formed blood vessels comprise 60% of the repair tissue. Neovascularization helps hypoxic wounds to attain the normoxic conditions. The CAM assay was done in prepared Polyherbal formulation, control and standard. As shown in the figure below the Prepared polyherbal formulation possess the good growth of blood vessels as compared to control and standard. This observation indicates that the developed veterinary polyherbal formulation has good angiogenic property. Improved angiogenesis, therefore, would be contributing significantly to wound healing activity of polyherbal formulation.

Keywords: CAM, Polyherbal formulation

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Received 29 November 2013, Accepted 02 December 2013

Please cite this article in press as: Shrimanker M. *et al.*, Preparation and *In-Vitro* Evaluation of Polyherbal Formulation for Wound Healing. American Journal of PharmTech Research 2013.

INTRODUCTION

Natural products are an integral part of human health care system now a days because there is now popular concern over toxicity and side effects of modern drugs. There is also a realization that natural drugs are safer and allopathic drugs are often ineffective. The WHO has also appreciated the fact that most of the world population depends on traditional medicine and therefore WHO has evolved guidelines to support the same.³In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects.

A wound can be described as a defect or a break in the skin, resulting from physical or thermal damage or as a result of the presence of an underlying medical or physiological condition¹. Wounds are the physical injuries that result in an opening or breaking of the skin and appropriate method for healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin¹ Healing of wounds starts from the moment of injury and can continue for varying periods of time depending on the extent of wounding and the process can be broadly categorized into three stages; inflammatory phase, proliferate phase, and finally the remodeling phase which ultimately determines the strength and appearance of the healed tissue² In other words, The wound healing process passes through four major phases such as hemostasis, inflammation, proliferation and remodeling³ The proliferative phase overlaps with the inflammatory phase. The most important cell is fibroblast which is responsible for initiating angiogenesis, epithelialization and collagen formation.⁴

MATERIALS AND METHOD

Materials:

The developed veterinary polyherbal formulation consists of *Curcuma longa*, *Glycyrrhiza glabra*, *Terminalia chebula* and Anthracinum 30 (A homeopathic preparation). All the mentioned crude dugs and homeopathic preparation were procured from the local market of Gandhinagar. All these plants were identified and authenticated by Dr. H. B. Singh, Taxonomist, NISCAIR, New Delhi. Voucher specimen of the plants (NISCAIR/2124/131) had been submitted in the institute for reference purpose. All other reagents are used of AR grade.

Preparation of wound healing formulation

Preparation of Sample Solution and combination of extracts:

Different concentrations of extracts using DMF were prepared. The ethanolic extracts of *Curcuma longa* and aqueous extracts of *Glycerrhiza glabra* and *Terminalia chebula* were taken

and alcoholic solutions were evaporated in order to make sure that the antibacterial activity solely contributes to the plant only. The extracts were taken in different ratio randomly and the antimicrobial tests were carried out for all the combination of extracts. The most effective combination was then determined by comparing the results of the Zone of inhibition. From the results the concluded ratio of the *C. Longa: G. glabra: T. chebula* was found to be 5:4:4.

Preparation of the ointment:

The ointment base was prepared by fusion method. In this method the constituents of the base were placed together in the basin and allowed to melt together at 70°C. After melting, the ingredients were stirred gently maintaining temperature of 70°C for certain periods and then at comparatively cooled temperature the herbal ingredients *C. Longa: G. glabra: T. chebula* (5:4:4) were added with continuous stirring.

***In-vitro* model for Wound Healing Activity**

Chick Chorioallontaic Membrane Assay

Chorioallontic membrane assay was used as *in vitro* model. This model was used to assess the angiogenic activity of polyherbal formulation. In this method, 9-day-old fertilized chick eggs were selected, and a small window of 1.0 cm was made in the shell. The window was opened, and a sterile disc of methylcellulose loaded with formulation was placed at the junction of two large vessels on CAM. The window is resealed by tape, and the eggs were incubated at 37°C in a well-humidified chamber for 72 h. Then, eggs were opened, and new blood vessel formation was observed in CAM treated by formulation which are compared with CAM containing disc without formulation (control) and the CAM treated with 10 µL 1000 AU/mL bFGF (Fibroblast Growth Factor) as a standard.⁵

RESULTS AND DISCUSSION

In general, Healing is normal physiological process that does not require but still take attention due to risk of infection & other severe complication. In this study, polyherbal formulation was selected on the basis of their reported proliferation of fibroblast growth factor which would help into new blood vessel formation. Angiogenesis plays an important role in wound healing and newly formed blood vessels comprise 60% of the repair tissue. Neovascularization helps hypoxic wounds to attain the normoxic conditions.

The polyherbal formulation showed an increased density of new blood capillaries and formation of microblood vessels on the treated membrane surface as compared to negative control as shown in Figure 1.

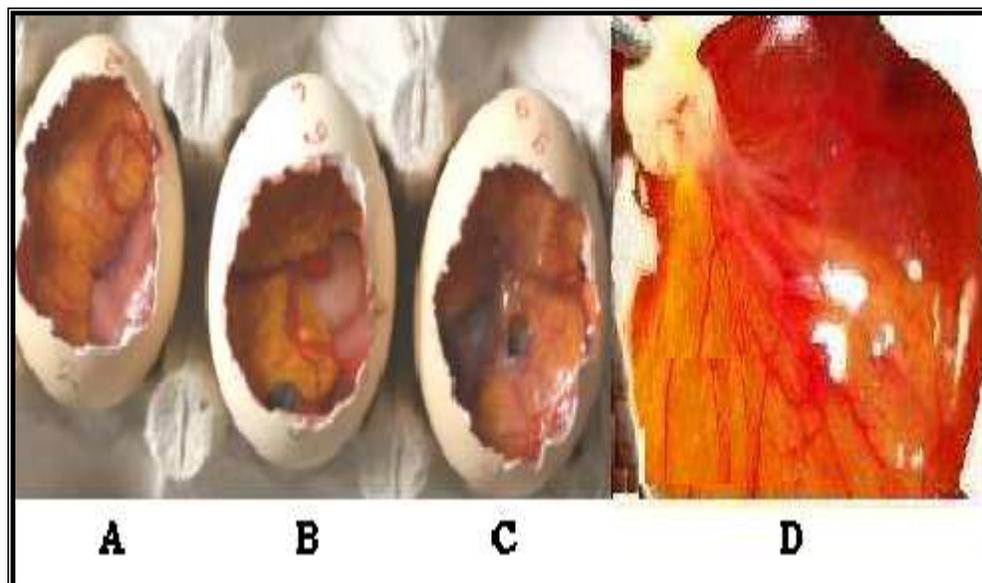


Figure 1: Where A = CAM treated with 0.9% NaCl (Control); B = CAM Treated with developed veterinary polyherbal formulation; C = CAM Treated with 10 µL 1000 AU/mL bFGF (Fibroblast Growth Factor); D = CAM Treated with developed veterinary polyherbal formulation showing blood vessels surrounded and attached to the disc.

Out of three eggs, two eggs from polyherbal formulation showed the attachment of disc with the new developing, budding blood capillaries with good density of new blood vessels surrounding the disc area such finding was absent from control group as shown in Figure 2

This observation indicates that the developed veterinary polyherbal formulation has good angiogenic property. Improved angiogenesis, therefore, would be contributing significantly to wound healing activity of polyherbal formulation.



Figure 2: New blood vessel generation in Control, Test and Standard sample

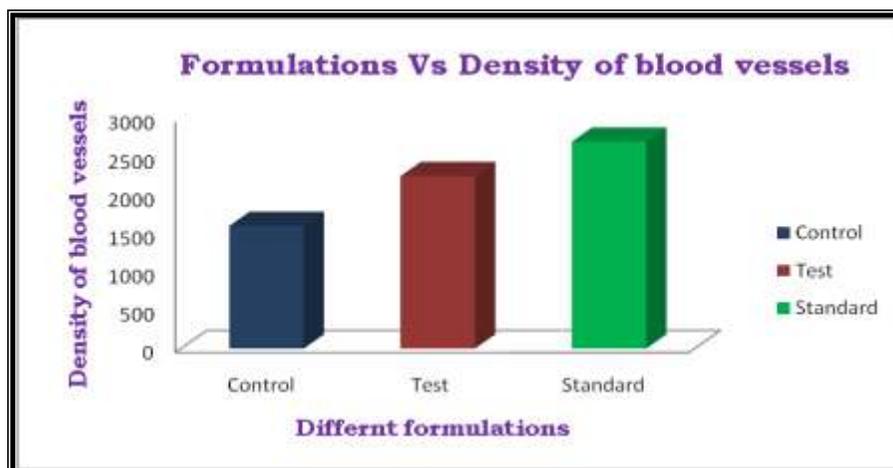


Figure 3: Graph of Formulations Vs Density of blood vessels

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