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DESIGN, FORMULATION AND EVALUATION OF A POLYHERBAL OINTMENT FOR ITS WOUND HEALING ACTIVITY

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ABSTRACT

Non-healing wounds are a significant problem in health care systems all over the world. Unlike other areas of health care, wound management need much attention for maximum benefit. During the last ten years different types of multidisciplinary concepts for the treatment of wounds have been created. A number of drugs ranging from simple non-expensive analgesics to complex and expensive chemotherapeutic agents administered in the management of wound. These drugs do not play major role in the wound healing process. The production and clinical evaluation of herbal remedies have made revolution in the use of traditional medicine with modern industrial venture rather than the use of raw herbal materials. On this basis, The present study was aimed at designing, formulating and evaluating a polyherbal ointment comprising of ethanolic extracts of the leaves of A*zadirachta indica,, Leucas zeylancia Ocimum sanctum ,rhizome of Curcumalonga juice of calotropis gigantean and Aloe vera* The parameters evaluated for wound healing were period of contraction and tensile strength using excision and incision models. The animals were divided into groups and were treated with polyherbal formulation, standard group and one served as control group. Nitrofurazone (0.2% w/w) was used as reference standard. The period of epithelization in the excision wound mode was found to be 13.50 days, while in case of incision wound model the tensile strength was 550 when compared to the controls ,the results were extremely significant. Different parameters like pH, viscosity, spreadability and stability were evaluated. The formulation showed good spread ability, good consistency, homogeneity, there was no change in the appearance, pH, and no phase separation noticed at the end of the stability studies. There was no evidence of skin irritation. This study has revealed that the poly herbal

ointment has shown the wound healing effect due to the synergistic activity of the phytoconstituents present in the extracts and may be used a potential herbal formulation for wound healing.

KEYWORDS: *Azadirachta indica, Leucas zeylancia Ocimum sanctum, rhizome of Curcumalonga juice of calotropis gigantean and Aloe vera*

INTRODUCTION

Herbal therapy predominates in traditional medicine as well as in alternative medicine practiced in the developing and the developed world. The widespread interest in drugs derived from plants is because of the belief that plants are safe and dependable, and with lesser side effects. Review of literature reveals that traditional plant drugs are beneficial for several skin related problems and for wound healing.[1] A poly herbal formulation consists of more then one herb. It is known that plants have different phytoconstituents which are responsible for the various activities that are attributed to them and when a combination of plants with these constituents are combined together it may show better activity when compared to the individual extract.[2] But at the same time presence of many constituents may lead to chemical incompatibility which may result in instability. Hence it is a challenging task to formulate a stable polyherbal formulation. Thus, the main objective of the present study is to formulate and evaluate a polyherbal formulation which may be used as a potent wound healing agent .In the present study an attempt has been made to formulate and evaluate a polyherbal formulation consisting of the extracts of A*zadirachta indica,, Leucas zeylancia Ocimum sanctum ,rhizome of Curcumalonga, juice of calotropis gigantean and Aloe vera* for its wound healing activity. These plants have been used traditionally for treating skin diseases, wounds and inflammation; hence an ointment prepared from these plants could be an effective treatment for different types of wounds. Wound is defined as the disruption of the cellular and anatomic continuity of a tissue; it may be produced by physical, chemical, thermal, microbial or immunological insult to the tissue.[1] Wound healing is the process ofrepair that follows injury to the skin and other soft tissues. Restoration of damaged tissue, wound or fracture is an important process which play a vital role in survival of life.[2] Wound care and maintenance involve a number of measures including dressing and administration of painkillers, use of anti-inflammatory agents, topical and systemic antimicrobial agents and healing drugs. The Indian traditional system of medicine described several drugs of plant,mineral, and animal origin is in the Ayurveda for their wound healing properties.[3]

More than 80% of the world's population still depends upon traditional medicines for various skin diseases.[4] Herbal medicines in woundmanagement involve disinfection, debridement and providing a moist environment to encourage the establishmentof the suitable environment for natural healing process.[5] *Tridax procumbens*, *Curcuma longa* and *Aloe vera* arethe three important herbs reported to have significant anti-bacterial, immunomodulatory and anti-inflammatory activities[6][-8], which are complementary to wound healing process. The management of wound healing is acomplicated and expensive programme, to rise above the situation use of natural and herbal medications with easy availability of raw materials encouraged us to formulate a polyherbal topical preparation and evaluate its wound healing ability in rats. The combination is used in order to enhance the wound healing activity

MATERIALS AND METHODS

Extraction of A*zadirachta indica,, Leucas zeylancia Ocimum sanctum ,Rhizome of Curcumalonga, Latex of calotropis gigantean and Aloe vera* Juice.

The dried powder of A*zadirachta indica,, Leucas zeylancia Ocimum sanctum* whereas the dried coarse powder of *Curcuma longa* rhizomes were extracted with ethanol by using soxhlet apparatus and the fresh *calotropis gigantean*, *Aloe vera* leaves juice were collected in beaker and was air dried. The extract was stored in desiccators for further study.

Preliminary Phytochemical Investigation.

The extracts of A*zadirachta indica,, Leucas zeylancia Ocimum sanctum ,Rhizome of Curcumalonga, Latex of calotropis gigantean and Aloe vera* Juice were subjected to various chemical tests For the phytochemical screening included the test for alkaloids, cardiac glycosides, terpenes, cyanogenic glycosides, carbohydrates, anthraquisnone, polyphenols saponins, tannins, flavonoids, phlobatanins, Resins and balsams.[9]

Preparation of emulsifying wax

All the ingredients mentioned below in the table-I were weighed accurately. The cetosteryl alcohol was melted at 90^{o}C the sodium lauryl sulphate was added and mixed properly further to this purified water was added. The container was heated at 115^{o} C with constant stirring till the product became transparent. It was further cooled and stored.[10]

Chemicals and Reagents

Emulsifying wax, white soft paraffin, liquid paraffin, methanol, agar Preparation of the Extracts. The extracts were prepared by macerating the powders of the leaves in methanol for

48 Hrs.Formulation of Ointment. The required quantity of the chemicals was weighed and the polyherbal ointment was formulated by fusion method using emulsifying ointment base.Evaluation of the Polyherbal Formulation. The polyherbal formulation was evaluated by the following physicochemical parameters.[14]

Colour and odour

Color and odor was examined by visual examination. Loss on drying Loss on drying was determined by placing the ointment in a petridish on a water bath and dried until constant weight was obtained.The pH of the formulation was recorded using a digital pH meter. Weighed quantity of the sample was dissolved in distilled water and stored for two hours. The measurement of pH was done in triplicate and average values were considered. Spreadability. The spread ability was expressed in terms of times in seconds taken by two slides to slip off from ointment placed in between the slides under the direction of certain load. Spread ability was calculated by using the formula.

$S = (M.L/T)$

Where, S = Spreadability, M = Weight tied to upper slide, L = Length of glass slides and T = Time taken to separate the slides.

Diffusion study

The diffusion study was carried out by preparing agar nutrient medium of known concentration. It was poured into a petridish and allowed to set. A hole was bored at the centre of the petridish and the prepared formulation was placed in it. The time taken for the ointment to get diffused was noted. Healthy rabbits were selected and were shaved in two different areas of the dorsal side, each about 500 mm^2.The rabbit was kept in rabbit holder and the first area was kept as control, to which emulsifying ointment base was applied, the second area was treated with polyherbal ointment. After 4hrs the skin was observed and compared with the control. Stability studies. The stability studies were carried out for the prepared Polyherbal formulation at different temperature conditions (4˚C, 27˚ C and 37˚C) for 3 months. Wound Healing Activity Was Carried Out Using the Following Models16.

Excision wound model

Male and female healthy wister albino rats weighing approximately 250-280 gs were used for the study. The animals were maintained under standard conditions during the course of the experiment. The animals were anesthetized using ether. An impression was made on the

dorsal thoracic region 1 cm away from the vertebral column and 5 cm away from the ear of the anesthetized rat. Skin was excised to full thickness to obtain a wound area of about 500 mm^2. The animals were divided into four groups and they were treated as follows: Group I: emulsifying base, Group II: standard nitrofurazone 0.2% (w/w) ointment, Group III: polyherbal formulation and Group IV: control .The ointments were applied once daily until complete healing of wound and the wound area was measured on a millimeter scale graph paper on alternate days. The percentage of wound healing was calculated. Falling of scar was taken as the endpoint for complete epithelization and the days taken for this was considered as period of epithelization.

Incision wound model

The selection of the animals was done as mentioned above. Two paravertebral straight incisions of 6 cm were made on either sides of the vertebral column. Homeostasis was achieved by blotting the wound with a cotton swab dipped in saline and the wound was closed by means of interrupted sutures at equidistance 1 cm apart. Animals were treated daily with formulations, as mentioned above under excision wound model from 0 day to 9th post-wounding day. The tensile strength in each group is determined on the 10th day by continuous, constant water flow technique.

RESULTS AND DISCUSSIONS

A wound may be defined as the loss or rupture of the cellular, anatomical or functional continuity of living tissue. Healing of skin wounds is a complex process which ultimately leads to the restoration of the injured skin. The aim of wound care is to promote wound healing in the shortest time possible[17] The process of wound healing is promoted by several natural products which have been reported and used in Ayurveda, Siddha and Unani systems of medicines.[18] These either promote direct wound repair or exhibit related properties like anti microbial, analgesic and anti inflammatory properties which are beneficial in overall wound care. Several phytoconstituents like phenolic compounds, flavonoids, saponins are known to promote wound healing process due to their anti-oxidant and anti-microbial properties.[19],[20] The results of this study have shown that the poly herbal formulation has shown significant activity when compared to the control and the base. The period of epithelization in the excision wound mode was found to be 13.50 days in case of the formulation when compared to control which was 19.00 days. While in case of incision wound model the tensile strength was 550 in case of formulation which is extremely

significant(**P<0.001). The formulation was evaluated for its physicochemical properties and was subjected to stability studies. These physical parameters were within the acceptable range and the formulation was found to be stable at the end of the stability studies i.e. 90 days. The extracts of these plants contain flavonoids and phenolic compounds. The activity of the polyherbal formulation can be attributed to the presence of these constituents.

Table1: Phytochemical Screening of the hydro-alcoholic extract of *Azadirachta indica,, Leucas zeylancia Ocimum sanctum ,rhizome of Curcumalonga, juice of calotropis gigantean and Aloe vera*

Constituents	*Azadirachta indica*	*Leucas zeylancia*	*Ocimum sanctum*	*Rhizome of Curcumalonga*	*Latex of calotropis gigantean*	*Aloe vera*
Carbohydrates	+	+	+	+	+	+
Proteins	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+

Table 2: Physiochemical properties of the formulation

Physicochemical parameters	Formulation
Colour	Dark green
Odour	Characteristic
Loss on Drying	10.6%
pH	6.7
Spreadability(Seconds)	16
Diffusion study	0.7cm
Skin irritation study	No skin irritation was observed
Storage(4^0,24^0,37^0 C)	Stable

Table 3: Effect of the polyherbal formulation in excision wound model

Treatment	50% wound contraction in days	Period of epithelization in days
Control	11.7±0.03	19.00±0.48
Standard	7.6±0.01	14.00±0.40*
Base	7.9±0.03	16.00±0.00
Formulation	7.1±0.2	13.50±0.02**

All values are mean SEM ±n=6, *P<0.05 indicates significant and**P<0.001 indicates extremely significant compared to the control.

Table 4: Effect of the polyherbal formulation in excision wound model

Treatment	Tensile strength
Control	310±9.36
Standard	450±13.00
Base	400±15.00
Formulation	550±9.5**

All values are mean SEM ±n=6, **P<0.001 indicates extremely significant compared to the control.

CONCLUSION

The results obtained in this study show that the combination of Tectona grandis, Ficus religiosa and Caesalpinia pulcherrima formulated as polyherbal ointment accelerates the healing process by enhancing collagen formation and increasing the breaking strength of the healed wounds. This potent activity can be attributed to the phyto-constituents present in the plants which may be acting synergistically to enhance the wound healing effect.

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