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TO STUDY THE WOUND HEALING ACTIVITY BY USING HERBAL DRUG ON EXPERIMENTAL ANIMAL

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ABSTRACT

A wound is a type of injury which occurs relatively quickly in which skin is torn, cut, or punctured (an open wound), or where blunt force trauma causes a contusion (a closed wound). Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury, an inflammatory response occurs and the cells below the dermis begin to increase collagen (connective tissue) production. Later, the epithelial tissue (the outer skin) is regenerated. There are three stages to the process of wound healing: inflammation, proliferation, and remodelling. The wound-healing efficacy of methanolic extracts of Cheilanthes albomarginata was evaluated in excision and incision wound models. The parameters studied include rate of wound contraction, period of complete epithelialization and tensile strength of incision wound. Student's t test was used to analyse the results obtained from the present study and P<0.05 was considered significant. Methanolic extract of Cheilanthes albomarginata was found to possess significant wound-healing activity, which was evidenced by decrease in the period of epithelialization, increase in the rate of wound contraction and skin-breaking strength. The present study has demonstrated that the methanolic extract of Cheilanthes albomarginata have properties that render them capable of promoting accelerated wound-healing activity.

***Key words:** Excision wound model, Cheilanthes albomarginata*

INTRODUCTION

There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine. It has very rich flora and fauna. There are about 17,500 species of flowering plants, 140 genera and 5285 species are native to the country. Many of these plants are used traditionally for treatment or cure of human and animal ailments. Most herbal drugs use leaves (34%) followed by bark (15%), entire plant (12% mostly in case of smaller herbs), underground plant parts (10% including tuberous, rhizome and bulb) and exudates like latex, gum or resin (8%). Lack of standard of efficacy and safety in traditional medicinal plants or herbal drugs is an issue. They are inconsistent in quality. They show various pharmacological

properties and phytochemical profile. Their active principles are not know. There are limited studies on traditional drugs with modern standards of safety and efficacy [1-3].

There are two approaches to develop successful drugs from medicinal plants: phytotherapeutic approach and the phytochemical approach. Then their pharmacological activities are evaluated and are practiced based on their chemical structure. Phytochemicals are chemicals extracted from plants. They may be primary or secondary metabolites. Primary constituents include the common sugars, proteins, aminoacids, purines and pyrimidines of nucleic acids, chlorophylls etc, that are essential for growth of metabolites of plants. Secondary metabolites are tannins, saponins, alkaloids, flavonoids, anthraquinones, cardiac glycosides, cyanogenic glycosides etc [4].

Wound is a disruption of the cellular and anatomic continuity of a tissue, with or without microbial infection and is produced due to any accident or cut with sharp edged things. It may occur due to thermal, chemical, physical, microbial or immunological exploitation of the tissues. If it is not treated immediately, it can lead to microbial infections. The wound infections are most common in developing countries due to poor hygienic condition. The microorganisms like gram positive and gram negative bacteria are the principal pathogen of wound infections. Diabetic patients are at increased risk of developing infection. This is due to impaired leukocyte function associated vascular diseases, poor glucose control and altered immune response [5].

Wound healing is a complex phenomenon for the regain or restoration of disrupted anatomical continuity and disturbed functional status of the skin, accomplished by several processes which involve different phases including inflammation, granulation, fibro genesis, neo-vascularization, wound contraction and epithelisation. The basic principle of optimal wound healing is to minimize tissue damage, provide adequate tissue perfusion, oxygenation, proper nutrition, moist wound healing environment. The main aim of wound therapy is to enhance wound healing in the shortest time possible, with minimal pain, discomfort and scarring to the patient and must occur in a physiologic environment conducive to tissue repair and regeneration [6].

Cheilanthes albomarginata belongs to family Pteridaceae found throughout India. Rhizomes are bluntly quadrangular, long and broadest in the middle, broader angle. Traditionally Rhizomes of Cheilanthes albomarginata are used in diseases of the blood, wound, ulcer, skin disorder, cuts. Etc. There are reports of antioxidant, anti-inflammatory, cytotoxicity, anti-arthritis, antimicrobial, antibacterial activity of Cheilanthes albomarginata [7].

So, the present is undertaken to evaluate the wound healing activity of Methanolic extract of Cheilanthes albomarginata on rats. Wound healing activity was evaluated by using excision and incision wound model in normal rats. The parameters used to access wound healing activity in this study are: Percentage wound closure of excised wound, tensile strength of incised wound [8].

MATERIALS AND METHODS

PLANT MATERIAL

The rhizomes of *Cheilanthes albomarginata* were collected during the month of December 2016 from Tirupati, Andra Pradesh, India. The plant material was taxonomically Identified and authenticated by Assistant Prof. K, Madhava Chetty, Plant Taxonomist, Shri Venkateswara Univarsity, Tirupati, India. A voucher number was 0985 [9].

EXTRACTION PROCEDURE

The powdered plant material was extracted using 95 % methanol and the solvent was completely removed by vacuum distillation to yield a residue 20.7 %, w/w respectively. This extract was examined chemically and was observed to contain flavonoids, terpenoids, and sterols. These constituents were confirmed using thin-layer chromatography (TLC).

ANIMALS

Albino (Wister) rats 180-200 g of either sex were used. The animals were kept in the standard polypropylene cages and provided with food and water ad libitum. The animals were acclimatized for period of 14 days prior to performing the experiments. The experimental protocols were approved by Institutional Animal Ethics Committee (Regn No: DYPIPSR/IAE/2016-17/P-07).

EXCISION WOUND MODEL

Four groups of animals containing six in each group were anaesthetized by open mask method with anaesthetic ether. The rats were depilated on the back and a predetermined area of 500 mm2 full thickness skin was excised in the dorsal interscapular region. The animals of group I were left untreated and considered as the control, group II served as reference standard and treated with 10 % w/w Betadine Ointment, animals of group III and IV were treated with ointment prepared from methanolic extract of *Cheilanthes albomarginata* (5 % and 10 %w/w). The progressive changes in wound area were monitored planimetrically by tracing the wound margin on a graph paper every alternate day. The change in healing of wound, i.e. the measurement of wound

area on graph paper was expressed as unit (mm2). Wound contraction was expressed as percentage reduction of original wound size [10].

INCISION WOUND MODEL

Four groups of animals containing six in each group were taken. The animals were anaesthetized under light ether anaesthesia. One full thickness Para vertebral incision of 6 cm length was made including the cutaneous muscles of the depilated back of each rat. Full septic measures were not taken and no local or systemic antimicrobials were used throughout the experiment. After the incision was made, the parted skin was kept together and stitched with sutures, 1 cm apart. The continuous threads on both wound edges were tightened for good adaptation of wound and it was left undressed. The ointment of the methanolic extract (5 % and 10 %w/w), standard drug (Betadine ointment) and simple ointment were applied to the wound twice daily, until complete recovery to the respective groups of animals [11].

RESULTS

The progress of the wound healing induced by *Cheilanthes albomarginata* rhizome extract ointments (5 % and 10 %w/w) treated groups, simple ointment (control) treated group and Betadine ointment (standard drug) treated group of animals are shown in Table 1. It is observed that the wound contracting ability of the extract ointment in different concentrations was significantly greater than that of the control (i.e. simple ointment treated group). The 10 % (w/w) extract ointment treated groups showed significant wound healing from the fourth day onwards, which was comparable to that of the standard drug, i.e. Betadine ointment treated group of animals. The wound closure time was lesser, as well as the percentage of wound contraction was much more with the 5 % w/w treated group (18 days for 100% contraction which was almost similar to that of the Betadine ointment treated group. *Cheilanthes albomarginata* (5 % w/w) ointment treated group of animals showed significant wound contraction from the sixth day onwards and achieved 100% with the wound closure time of 19 days.

Table1: Evaluation of MECA extract on wound healing by excision wound method in rats.

Group dose	**0th day**	**2nd day**	**4th day**	**8th day**	**12th day**	**16th day**	**18th day**	**20th day**
Control	501.5 ±3.28	455.7±1.67 (9.15%)	483.2±3.22 (23.54%)	301.6±2.30 (39.66%)	235.2±2.25 (52.89%)	131.7±2.75 (73.20%)	67.32±1.56 (87.40%)	24.62±1.52 (95.06%)
Beadine Ointment	501.6±2.206	444.6±4.67 (11.33%)	322.7±3.21 (35.63%)	171.2±3.53 (65.62%)	101.3±2.14 (79.52%)	18.32±1.12(95.24%)	- (100%)	- (100%)
MECA (5% w/w)	501.1±3.22	444.7±4.66 (10.66%)	346.1±2.41 (26.91%)	218.7±2.66 (55.6%)	134.2±2.21 (72.12%)	74.3±2.51 (84.5%)	9.6±1.22 (97.66%)	- (100%)

MECA (10%w/w)	501.6±2.43	447.1±2.54 (10.95%)	344.5±3.19 (31.99%)	207.9±4.09 (57.45%)	119.1±3.11 (77.11%)	23.22±1.19 (94.46%)	- (100%)	- (100%)
Note: Wound area (mm2) mean ± SEM and percentage of wound contraction.								

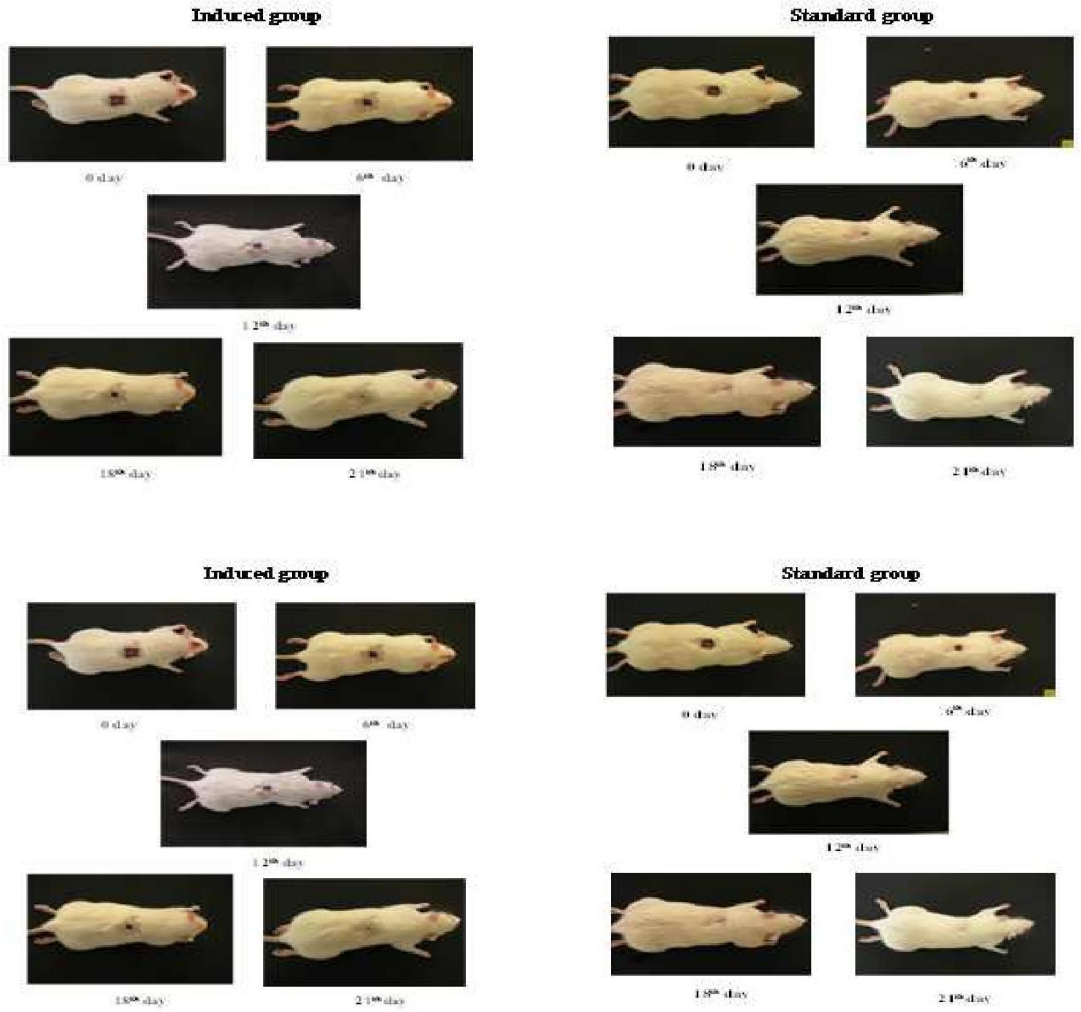

Figure1: Evaluation of MECA extract on wound healing by excision wound method in rats.

INCISION WOUND MODEL:

The measurement of the effect of the extract and standard drug on the tensile strength of the incision wound is shown in Table 2. The tensile strength of the MECA (5 % and 10 % w/w), extract treated group and the Betadine treated group were comparable to

each other. Thus both concentrations of the extract as well as the standard drug showed a significant increase in tensile strength in the 10 days old wound.

Table 2: Evaluation of MEIF extracts and standard drug on incision wound model in rats.

Treatment	Excision wound tensile strength (g) (mean ± SE)	Incision wound Epithelialisation period (days)
Control	418.4 ±1.63	23.1 ±1.26
10% betadine ointment	548.3 ±1.78**	18.6 ±0.87**
MECA (5% ointment)	494.6 ±1.87**	17.3 ±0.83
MECA (10% ointment)	527 ±1.42 **	17.4 ±141**
Note: All values are represented as mean ± S.E.M. (n = 6). ANOVA followed by Dunnett's't' test. *P < 0.05, **P < 0.01 when compared to control.		

Discussion

Wound healing is the physiological response to the tissue injury that results in the replacement of destroyed tissue by living tissue and thus restoration of tissue integrity. The mechanism of wound repair occurs by four basic processes such as inflammation, wound contraction, epithelialisation and granulation tissue formation. Inflammation starts immediately after the disruption of tissue integrity. The platelets became adherent with clotting factors and form haemostatic plug to stop bleeding from the vessels. The prostaglandins are released in the inflammation area and seem to be the final mediators of acute inflammation and may play a haemostatic role for white cells and fibroblasts. The active motile white cells migrate into the wound and start engulfing cellular debris, at the initial stages wound contraction begin slowly but became rapid after 3 or 4 days. The myofibroblsts present in the margin of the wound appear to constitute the machinery for the wound contraction. These are responsible for overlaying debris. The epithelialisation of the wound mainly occurs by proliferation and migration of the marginal basal cells lying close to the wound margin. The hematoma within the wound may be replaced by granulation tissue, which consists of new capillaries and fibroblasts. The fibroblasts are responsible for production of the mucopolysaccharide ground substance. The lymphatics develop new nerve fibres and there is also formation of scar tissue in which collagen turn over increases.

Preliminary phytochemical analysis revealed the presence of flavonoids, terpenoids and steroids. Flavonoids are known to reduce lipid peroxidation by preventing /slowing onset of cell necrosis as well by improving vascularity and drugs that inhibits

lipid peroxdiation is also believed to increase viability of collagen fibers by increasing the strength of collagen fibers, the circulation, preventing the cell damage and by promoting DNA synthesis. Flavonoids and terpenoids have been reported to promote wound healing due to its astringent and antimicrobial property, which may be contributing to wound contraction and increase rate of epithelialisation. Antioxidant property of flavanoids and terpenoids may also be contributing to wound healing. The wound healing potential of the *Cheilanthes albomarginata* extract may probably be as a result of the presence of a mixture of phytoconstituents including flavonoids, terpenoids, steroids, etc., the isolation of which is under way in our laboratory. Thus from this study it is concluded that the *Cheilanthes albomarginata* rhizome extract has a reproducible wound healing potential and new skin generation mechanism and thereby justifies its use in folklore medicine in India.

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