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EVALUATION OF WOUND HEALING ACTIVITY OF ETHANOLIC LEAF EXTRACT OF *HELIOTROPIUM INDICUM* IN RABBIT BY EXCISION WOUND MODEL

B.Sangeetha[1]*, Palaksha M.N[2], Ravishankar K[2]

1. Student, Aditya College of Pharmacy, Surampalem, E.G.Dist-533437

2. Department of pharmacology, Aditya college of Pharmacy, Surampalem, E.G.Dist-533437.

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For Correspondence:

B.Sangeetha

Aditya College of Pharmacy, Surampalem, E.G.Dist-533437

ABSTRACT

The present study was to evaluate the wound healing activity of ethanolic leaf extract of *Heliotropium indicum* in rabbit by excision wound model. In this study, the leaf of *Heliotropium indicum* was collected from the surrounding villages of Divili East Godavari district. The dried plant powder was extracted with ethanol. The wound was made by excising the skin, within the border of the template to the level of loose subcutaneous tissue, using a size No.15 scalpel blade and a forceps. The wound closure time was lesser, as well as the percentage of wound contraction was much more with dose of extract and 100% *Heliotropium indicum* contraction was observed in (15.7±0.14) days, which was almost similar to that of betadine treated group (14.8±0.6) days. The 5% extract group of animals showed significant wound contraction from 6th day onwards and achieved 100% wound closure in 18.9±.38 days. The study provides scientific evidence for further evaluation of *Heliotropium indicum* in the topical treatment and management of wounds.

INTRODUCTION

Wound healing is a normal biological process in the human body that includes three overlapping phases: inflammation, tissue formation, and remodeling. It involves soluble mediators, blood cells, parenchymal cells, and extracellular matrix (A.F. Falabella et al., 2005)[1]. As the blood components fall into the injury site, the platelets move toward into contact with exposed collagen. This result, the platelets releases clotting factors and essential growth factors. In hemostasis, the neutrophils go in to the wound site and begin phagocytosis to remove bacteria, foreign materials, and damaged tissue. The macrophages appear in the inflammatory phase and continue the phagocytosis process. Once the wound site is cleaned out, fibroblasts migrate to start the tissue formation and deposit new extracellular matrix. The new collagen matrix organized by cross-linking during the final remodeling phase (R.F. Diegelmann et al., 2004)[2]. Due to poor hygienic condition and infection of pathogenic micro-organism creates difficulties to manage wound infection and presently wide range of antibiotics are being used for treating wound infection but due to their adverse effect and antibiotic resistance is now paying attention towards natural biologically active herbal compounds as an alternative medicine. (P. Mertz et al., 1993[3]; T. Essawi et al., 2000[4] ; L.R. Peterson ., 2005[5] ; M.E. Falagas et al., 2007[6]). Herbal medicines are being used by about 80% of the world population for primary health care due to their efficacy, safety, cultural acceptability, and less side effects. The plant constituents are a part of the physiological function of living flora and hence they have better compatibility with the human body (V.P. Kamboj., 2000)[7].

Heliotropium indicum Linn., (Family: Boraginaceae) commonly known as 'Indian heliotrope' is very common in India with a long history of traditional medicinal uses in many countries in the world. An ethnopharma-cological survey revealed that, the traditional healers in Kancheepuram district of Tamil Nadu, India use *Heliotropium indicum* to cure skin diseases, poison bites, stomachache and nervous disorders (Chellaiah et al., 2006)[8]. Heliotropium indicum is believed to be useful in treating malaria, abdominal pain and dermatitis (Togola et al., 2005)[9]. The decoction of the entire plant is taken orally for treatment of intractable fever, ulcers, venereal diseases and sore throat and used externally in vaginal cavity to induce abortion in pregnant females and administered rectally to treat local sores in the rectum and orally as diuretic and for the treatment of kidney stone (Berhault, 1974)[10].

The present study was to evaluate the Wound healing activity of ethanolic leaf extract of *Heliotropium indicum* on rabbit.

MATERIALS AND METHODS

Collection of plant:

The whole plant of *Heliotropium indicum* was collected from the surrounding villages of Divili East Godavari district. The plants were identified and authenticated by the taxonomist Mr.T.V.Raghavarao, Department of Botany SRVBSJB Maharanee College, Peddapuram, E.G.Dist. Andhra Pradesh.

Preparation of extract:

The freshly collected leaf of the plant were cleared from dirt and dried under shade and then coarsely powdered manually. The dried plant powder was macerated in ethanol for a period of 7 days and later subjected to hot percolation for 8 hrs. Then the solution was filtered, concentrated and subjected to drying. 100gm powder yields 4.5gm extract.

Experimental Animals:

Animals: Rabbits of either sex weighing 1.6-2.2kg were used during the study. Before and after surgery the animals were housed individually in iron cages cages. They were allowed to feed on a standard, commercial pellet diet supplemented with fresh vegetables and water ***ad libitum***. The animals were maintained in a holding room illuminated with 12 h light/dark cycles. Room temperature was set at 23±2°C with humidity of 45% to 55%. Hairs of lower back and left flank of the test animals were fully shaved and cleared, the desired area was locally sterilized and anaesthetized with the subcutaneous injection of 2% lidocaine (Sharma S 2009)[11].

A full thickness of the excision wound of circular area 500 mm^2 and 0.2 cm depth was created. The wound was made by excising the skin, within the border of the template to the level of loose subcutaneous tissue, using a size No.15 scalpel blade and a forceps. Wounds of animals were divided into 4 groups of 3 each. Group 1were treated topically with povidone iodine ointment (betadine) as a standard healing agent (Krishna Murthy 2012)[12]. Group 2 were treated as control group were treated with simple ointment base and Animals of groups 3 and 4 were treated with ethanolic extracts of Heliotropium indicum two doses concentration of the extract were prepared *viz*. (20% w/w) ointment, where 20g. Of extract was incorporated in 100g Of simple ointment base and (10% w/w) ointment where, 10g Of extract incorporated in 100g.of simple ointment base respectively (Ilango. K 2010)[13].

Changes in wound area were measured regularly and the rate of wound contraction was calculated as given in the formula below.

% wound contraction=100- [Final diameter (cm) ×100/initial diameter (cm)]

Significance in wound healing of the test groups is derived by comparing healed wound area on respective days with the control groups. The period of epithelisation was noted down (Nakae H etal., 2000)[14].

RESULTS

The wound healing contracting ability of ethanolic leafextract of *Heliotropium indicum* in different concentrations on excision wound model was significantly greater than that of the control group. The 10% extract ointment treated groups showed significant wound healing from 3rd day onwards which was comparable to that of standard drug i.e. Betadine treated group. The wound closure time was lesser, as well as the percentage of wound contraction was much more with dose of extract and 100% *Heliotropium indicum* contraction was observed in (15.7±0.14) days, which was almost similar to that of betadine treated group (14.8±0.6) days. The 5% extract group of animals showed significant wound contraction from 6th day onwards and achieved 100% wound closure in 18.9±.38 days as shown in table 2. The results of present study revealed that both concentrations (5% extract ointment, 10% extract ointment) of ethanolic extract of shoot tips have *Heliotropium indicum* significant wound healing activity in excision model.

TABLE NO.1 PERCENTAGE OF WOUND HEALING SHOWN BY ETHANOLIC EXTRACT OF *HELIOTROPIUM INDICUM* LEAFIN EXCISION WOUND MODEL:

Group	Treatment	Percentage (%) of wound healing on the day						
		3rd day	6th day	9th day	12th day	15h day	18th day	21st day
I	Control	2.67±0.16	5.6 ±0.17	30.8 ±0.21	48 ±0.22	54.7 ±0.13	72.8 ± 0.15	82.5 ±0.20
II	*Heliotropium indicum*(10% w/w extract ointment)	0.54±0.09	14 ±0.14	36.5 ±0.37	50.6 ±0.25	60.2 ±0.2	77.7 ± 0.35	91.5 ±0.18
III	Heliotropium indicum(20% w/w extract ointment)	1.39±0.13	18.16 ±0.08	40 ±0.15	57.7 ±0.18	65.7 ±0.23	80.8 ±0.15	93.9 ±0.18
IV	Standard (betadine)	2.69±0.20	22.50 ±0.28	44.7 ±0.19	58.9 ±0.10	67.5 ±0.32	88.7 ±0.16	96.3 ±0.18

Values are expressed as mean ±SEM;n=3 animals in each group;p≤0.001 when compared to control.

TABLE NO.2: EFFECT OF TOPICAL APPLICATION OF ETHANOLIC EXTRACT OF *HELIOTROPIUM INDICUM* LEAFON HEALING OF EXCISION WOUND MODEL:

GROUP	Post wounding days								Period Of Epithelialisation
	0 day	3rd day	6th day	9th day	12th day	15th day	18th day	21st day	
Control	2.48 ±0.11	2.45 ±0.01	2.34 ±0.07	1.73 ±0.09	1.33 ±0.09	1.17 ±0.08	0.75 ±0.08	0.53 ±0.1	25±1
H.indicum (10%w/w ointment)	2.82 ±0.33	2.41 ±0.05	2.17 ±0.13	1. 7 ± 0.2	1.25 ±0.07	1.08 ±0.15	0.64 ±0.1	0.30 ±0.11	23.6±1.5
H. indicum(20%w/w extract ointment)	2.43 ±0.07	2.38± 0.03	2.01 ±0.07	1.51 ±0.08	1.11 ±1.10	0.96 ±0.14	0.53 ±0.07	0.25 ±0.09	22±1
Betadine	2.44 ±0.07	2.36 ±0.04T	1.93 ±0.10	1.41 ±0.10	1.08 ±0.12	0.88 ±0.10	0.42 ±0.13	0.14 ±0.07	19±1

*Values are expressed as mean ±SEM;n=3 animals in each group;

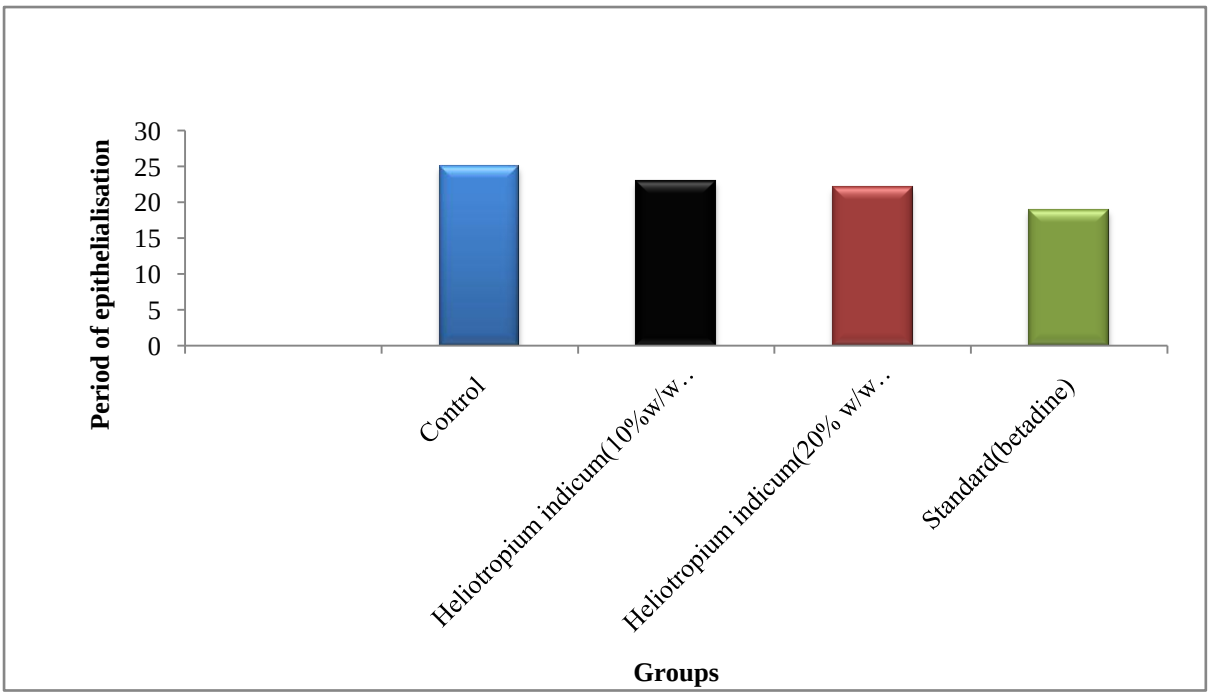

Fig No.1.Measurement of period of epithelialisation in excision wound model

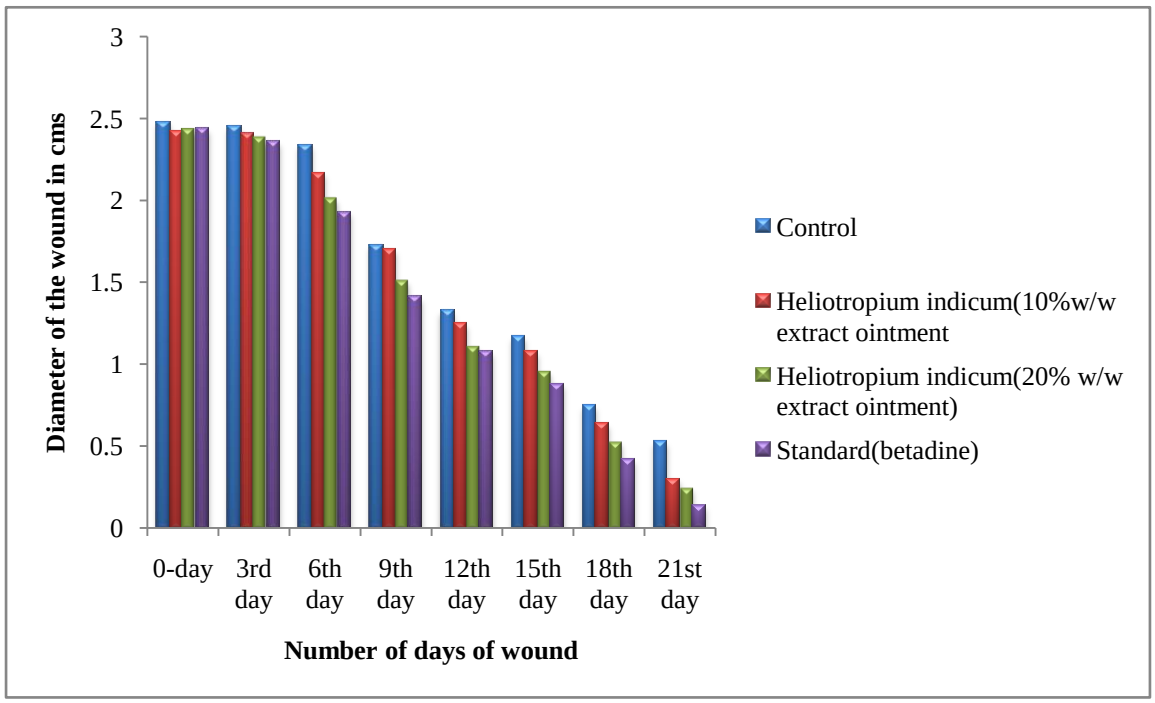

Fig No. 2. Measurement of wound diameter in excision wound model

DISCUSSION

The present investigation describes the wound healing effect produced by ethanolic extract ointment of leafextract of *Heliotropium indicum* (10% and 20%). Though the advances in synthetic medicine had replaced the herbs usage, the herbs are still being used among tribal people for curing various body ailments. The wound healing activity of the ethanolic leafextract of *Heliotropium indicum* was related to their chemical composition. The preliminary Phytochemical components of the *Heliotropium indicum* were include flavonoids, tannis, Saponins and alkaloids. This plant was having good antimicrobial activity; these actions in turn prevent the growth of microbes in wound area and thereby produce prominent wound healing effect. The constituents were extracted by using ethanol and the observations had shown that percentage wound reductions in test compound treated groups are more prominent than that in standard treated group. The usage of ointment preparation of extracts will decreases the unwanted systemic side effects and makes the drug readily available at site of action. The ointment shows rapid epithelisation time than others.

The healing of wound is a complex process that involves the activation and synchronization of coagulatory and inflammatory events, epithelialization, fibrous tissue accretion, deposition of collagen, wound contraction, tissue granulation and remodeling (P.K. Ghosh etal.,2013)[15] . Healing process takes place by immunological activities of victim itself and does not require

much help, but various risk factors such as infection and week immunity delay in healing has brought attention to promote this process.(S.K. Muthusamy etal., 2008)[16] and (B.S. Nayak etal.,2007)[17]

Wound contraction indicates the rate of reduction of the unhealed area during the healing process. Thus, a fast rate of wound contraction indicates better efficacy of medication. Wound contraction plays an important role in the closure of full thickness wounds, where the surrounding skin is pulled in by forces that develop within the granulation tissue (M. Ganeshkumar et al., 2012)[18]

CONCLUSION

The present study demonstrated that the ethanolic extract of *Heliotropium indicum* have properties of promoting accelerated wound-healing activity compared with control. The study provides scientific evidence for further evaluation of *Heliotropium indicum* in the topical treatment and management of wounds.

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