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"WOUND HEALING ACTIVITY OF *FOENICULUM VULGARE* IN SPRAGUE DAWLEY RATS."

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ABSTRACT

The study was conducted to determine the wound healing action of aqueous extract of *Foeniculum vulgare* On Sprague Dawley rats using excision wound model. The plant was collected and authenticated. The phytochemical investigation was done to investigate the presence of various components. 2% and 7% aqueous extract was used for determining the wound healing action. Vaseline was used as control while Mupirocin was used as standard. Post treatment the % wound contraction and wound area was measured on 4th, 8th, 12th and 16th day. the results suggested significant decrease in wound area and Change in % wound contraction was significantly observed.

KEYWORDS: *Foeniculum vulgare,* excision wound model, wound area, %wound contraction.

INTRODUCTION

Wound healing refers to the restoration of continuity of living tissue. Wound healing is not an isolated single phenomenon, but a complex series of biological events, the end result of which is preservation of life. Injury to tissue may result in cell death and tissue destruction. Healing is the body's response to injury in an attempt to restore normal structure and function.[1]

There are 3 to 5 stages of wound healing, depending upon the various biological mechanisms are linked. The first stage, hemostasis occurs immediately at the time of injury and is usually completed within few hours. The second stage, inflammation begins shortly after hemostasis and is usually completed within the first 24 to 72 hours after injury; however, it may last as long as 5 to 7 days after injury. Proliferation and repair, the third stage typically occurs 1 to 3

weeks after injury. The fourth and final stage, remodelling begins approximately 3 weeks after injury and may take months to several years to achieve physiologic completion.[2]

Foeniculum vulgare Gaertn. Family Umbelliferae is a common herb that grows in many countries especially in the Mediterranean region.[3] Fennel and its preparations are used to cure various disorders, and also act as a carminative, digestive and diuretic agent. Fennel increases elasticity of connective tissues and act as anti-aging agent. There are some commercial pharmaceuticals with formula based on fennel essential oil. Fennel is also recognized to have anti inflammatory[4], antimicrobial and antioxidant properties.[5,6]

MATERIALS AND METHODS

Collection of plant

Fully riped seeds of fennel (*Foeniculum vulgare*) were collected from the local market (vanaushadhalay), Pune, Maharashtra during the month of December 2012. The seeds were then authenticated by the Agharkar Research Institute, Pune.

Plant extraction and standard used

The seeds were shade dried. The seeds were powdered and extracted with distilled water by keeping it for 7-8 days. The extract was then filtered off and filtrate was evaporated to dryness to obtain aqueous extract. Two types of ointment formulations with different concentration of the extract were prepared viz. 2% (w/w) ointment where, 2 gm of extract was incorporated in 100 gm of simple ointment base; 7% (w/w) ointment where, 7 g of extracts of the leaves were incorporated in 100 g of pure white petroleum jelly (Vaseline) obtained from Hindustan lever ltd, Andheri (E) Mumbai, Maharashtra. The standard used was Bactroban ointment 2% (mupirocin) manufactured by Glaxosmithkline Inc. for comparing the wound healing efficacy of the test drug used.

Animals: Sprague Dawley rats of either sex weighing between 150-250 gm were used for the experiment. Animals were obtained from National Institute of Biosciences, Pune. All the protocols were approved by Institutional animal ethics committee (SCOP/IAEC/Approval/2012-13/31), constituted for the purpose of control and supervision of experimental animals (CPCSEA) by Ministry of Environment and Forests, Government of India, New Delhi, India. Animals were housed into groups of six in polypropylene cages containing husk as bedding material and maintained under controlled conditions of temperature (23±2^{o}C), humidity (55±5%) and 12 h light and dark cycle in the animal house

of Sinhgad College of Pharmacy, Pune, India. The animals were fed with standard diet and water *ad libitum*.

Acute dermal toxicity test (limit test)

The test was performed on rats as per OECD guidelines 404 with modification. Hair was clipped off from back of the rats one day prior to the study. Animals were housed individually in cages in normal laboratory conditions and were provided with standard animal feed along with water ad libitum. Next day the different concentrations of the test ointment were applied on intact as well as abraded skin of different rats. Aqueous 0.8% solution of formaline was applied as standard irritant. These animals were observed for a period of seven days for development of any signs of oedema and erythema.[7,8]

Excision wound model

Animals were anaesthetized with 80 mg/kg dose of ketamine (i.p.) and hair on the back of animal were depilated by shaving. The back of the animals, in the cervical region of each animal, were surgically prepared for aseptic surgery. An excision wound of size 4 cm^2 was made by cutting out a 2×2 cm piece of skin from the shaved area. The wounds were of full thickness extending up to the subcutaneous tissue. The progressive changes in wound area were monitored planimetrically by tracing the wound margin on graph paper every alternate day. The wound induced day was considered as day 0. Wounds were treated with topical application of the ointment preparations till the wounds were completely healed. The wounds were monitored and the area of wound was measured on 4, 6, 8, 10, 12, 14, 16 post-wound induced days. The mean % wound closure was reported.[8-11]

For the purpose of study the animals were divided into four groups.

Group 1: Received treatment with only vehicle (Vaseline).

Group 2: Received treatment with fennel extract ointment (2%).

Group 3: Received treatment with fennel extract ointment (7%).

Group 4: Received treatment with standard ointment (Mupirocin 2%).

RESULTS

Phytochemical investigation of plant extract-

Evaluation of aqueous extract of *Foeiculum vulgare*

CONSTITUENTS	
SAPONINS	+
TANNINS	+
GLYCOSIDES	+
ALKALOIDS	+
FLAVONOIDS	+

Effect of *Foeniculum vulgare* on % wound contraction

A significant increase in % wound contraction was found in ointment treated groups compared to control group.

Table no:1 - Effect of *Foeniculum vulgare* on % wound contraction

Groups	4th day	8th day	12th day	16th day
I- Control	15.46±2.71	32.28±2.29	60.53±1.60	76.4±1.35
II-2% Ointment	24.26±1.40*	45.26±2.32*	66.46±1.94*	81.38±1.20*
III-7% Ointment	37.58±2.13*	55.78±1.55*#	81.38±1.30*	90.7±0.74*
IV-Standard	39.63±2.20*	58.83±2.06*	81.66±0.72*	92.05±0.50*

All values are expressed as mean ± SEM, n=6,

*p<0.01 when compared to normal control, #p<0.05 when compared with standard.

Statistical analysis was done by using two way ANOVA followed by Bonferroni test.

Effect of *Foeniculum vulgare* on wound area

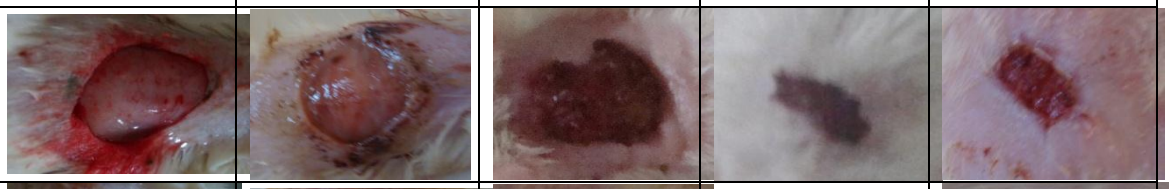
The area of wound contraction by Vaseline, *Foeniculum vulgare* 2% ointment, , *Foeniculum vulgare* 7% ointment, and Mupirocin cream treated animal group is shown in table : 1.

From the table we can observe that treatment by ointment in different concentrations showed better wound contraction when compared to control group and almost similar to the standard preparation.

Table no: 2 Effect of *Foeniculum vulgare* on wound area contraction.

Groups	4th day	8th day	12th day	16th day
I-Control	3.38± 0.109	2.709±0.092	1.579± 0.064	0.944± 0.05
II-2% Ointment	3.029± 0.056*	2.189± 0.093*	1.341± 0.078*	0.745± 0.048*
III-7%Ointment	2.497± 0.086*	1.769± 0.062*#	0.745± 0.052*	0.372± 0.030*
IV-Standard	2.413± 0.088*	1.647± 0.083*	0.733± 0.029*	0.318± 0.020*

All values are expressed as mean ± SEM, n=6,

GROUP	DAY 0	DAY 4	DAY 8	DAY 14	DAY 16
I					
II					
III					
IV					

*p<0.01 when compared to normal control, #p<0.05 when compared with standard.

Statistical analysis was done by using two way ANOVA followed by Bonferroni test.

CONCLUSION

The phytochemical investigation of aqueous extract showed the presence of tannins, saponins etc. *Foeniculum vulgare* showed significant reduction in the % wound contraction and the wound area in the 16 day study protocol. Thus, *Foeniculum vulgare* has a wound healing activity. Further studies on isolation and formulation are under consideration in the same laboratory.

DISCUSSION

Nowadays, many herbal plant products are being used and are found to be beneficial for treatment of various wounds. Medicinal plants have great potential and are beneficial in wound contraction. Overall performance of all the plants are not similar to each other. Each of them act by different mechanisms.The main aim of the wound healing is contraction of wound by promoting rate of wound healing with minimum pain, discomfort and scarring to patient.

Foeniculum vulgare is already noted to have anti-inflammatory property the study showed that, Ointment form shows wound healing property.in 2% and 7% concentration. The plant may be acting to reduce inflammatory phase and also reduce the chances of reoccurrence of

infection and pus formation due to its antibacterial and antioxidant property. The extract also shows presence of tannins which may contributes to the wound healing action.

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