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WOUND HEALING ACTIVITY OF LEAVES & ROOTS OF CALENDULA OFFICINALIS LINN OF BY USING DIFFERENT EXTRACTS

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ABSTRACT

Calendula officinalis which is knows as kankara in hindi, widely distributed in greater part of india. Calendula officinalisis a traditional medicinal plant having for various diseases. **Objective**: The aim of the present study to evaluate the wound healing activity on different extracts (Petroleum ether, Chloroform & methanol) of leaves & roots of Calendula officinalislinn in albino rats by using excision and incision wound models. **Method:** The excision and incision wounds were inflicted upon eight groups, each group having six albino rates and divided one group for control, 6 group for test and 1 group for standard treated with Povidin Iodine ointment. The parameter in Excision observed was wound contraction and Incision model was Tensile strength. **Result:** It was noted that the effect produced by the methanolic extract of calendula officinalis Ointment showed significant healing in both wound models. the studies indicate that the root extracts of plant having more potent healing as compared to leaves extracts. **Conclusion:** The results further suggest that *Calendula officinalis* facilitates healing by increasing the rate and extent of wound closure and increased tensile strength in wounds subject to healing.

1. INTRODUCTION

Nature was the main source of drug in ancient times, the progress and development of the human race is inseparably linked to the increasing scientific knowledge, man has been able to obtain over the centuries. Green plants are essential for all animal life on earth, since they convert solar energy into organic carbon compounds which is used as a basic energy for animals(1). A wound is a type of injury which happens relatively quickly in which skin is torn, cut, or punctured (an open wound), or where blunt force trauma causes a contusion (a closed wound). In pathology, it specifically refers to a sharp injury which damages the dermis of the skin. Wound infection is one of the most recurrent diseases in developing countries because of poor hygienic conditions. Wound is a break in the epithelial coherence of the skin and maybe leading by disruption of the structure and function of underlying normal tissues and may also result from a hematoma, contusion, laceration or an abrasion. Healing of wounds begins from the moment of injury and can be extended for varying periods of time depending on the degree of wounding and the process of wound healing can be broadly classified into three stages; inflammatory phase, proliferate phase, and finally the remodeling phase which finally governed the strength and appearance of the healed tissues (2). Four main phases of wound healing are hemostatisis, inflamamatory, proliferative and maturation phase (3).Calendula officinalislinn belonging to family Rubiaceae is shurb. In literature it has been reported as an diuretic activity (4), antinflamatory activity (5), antimicrobial activity (6), antidibetic activity(7), Analgesic activity (8), hepatoprotective (9), antioxidant activity (10) and anticorrosive nature (11).

2. MATERIAL AND METHOD

Plant Material

The leaves & Roots of the plant *Calendula officinalislinn* were collected from Bhopal (Vindya herbal medicinal garden) during the month of September - October. The collected material is compared with the published description of the drug and identification is verified by an acknowledged expert. The plant material was identified and authenticated by Dr. Ziaul Hasan, HOD, Department of Botany, Safia College of Science and Education Bhopal (MP) India.

Extraction of Plant Material

Extraction of plant material was done by successive solvent extraction method using various organic solvent as according to non-polar to polar (12). The plant material was placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhlet extractor. This extractor is placed upon a distillation flask containing the solvent. The Soxhlet is then equipped with a condenser, and the solvent is heated to reflux. The warm solvent vapor travels up a distillation arm and floods into the chamber housing the thimble. When the chamber is almost full, it gets automatically emptied by a siphon side arm back down to the distillation flask. This cycle may be allowed to repeat many times so that the desired compound gets concentrated in the distillation flask. The plant material was defatted with petroleum ether (40-

60°C) for about 09 hrs & complete defatting was ensured by placing a drop from the thimble on a filter paper which did not exhibited any oily spot. The defatted material was removed from the soxhlet apparatus and air dried to remove last traces of petroleum ether. The defatted material was subjected to extraction by chloroform and ethanol as solvent. The process was carried out for about different timings. for different solvents. The liquid extracts were collected in a tarred conical flask. The solvent was removed by distillation. Last traces of solvent being removed under vacuum. The extract obtained with each solvent was weighed to a constant weight and percentage w/w basis was calculated.

Wound Healing Activity

Selection of model

Excision and Incision wound model, using albino rats was selected for assessing the wound healing activity. This model was employed to study the rate of wound contraction, time and tensile strength estimation. These parameters were selected because of easy availability of albino rats and simplicity in handling them.

Selection and procurement of animals

Excision wound Model

In the excision wound model, rats were depicted by removing hairs at the dorsal thoracic region before wounding. Rats were anesthetized by diethyl ether prior to excision. Circular wound of about 2.5 cm diameter was made on depicted dorsal thoracic region of rats under aseptic conditions and were observed throughout the study. The areas of the wounds were measured (in mm2) immediately by placing a transparent polythene graph paper over the wound and then tracing the area of the wound on it (Approx. area 500 mm2). This was taken as the initial wound area reading (13).

The rats are categorized into seven groups (n=6). The animal of group I was treated as control and only ointment base applied topically. The animal of group II, III and IV were treated with ointment of pet ether, chloroform and ethanol leaves extract of *Calendula officinalis* and group V, VI and VII were treated with ointment of pet ether, chloroform and ethanol roots extract of *calendula officinalis.*

All the samples were applied topically once daily for 16 days, starting from the day of wounding. The observations of percentage wound closure were made on 4th, 8th, 12th and 16th, post wounding days. The wound area of each animal was measured by using tracing paper method. The percentage of wound contraction was calculated from the days of measurements of wound area.

Wound Contraction

The wound contraction was calculated as percentage reduction in wound area with respect to initial wound area while the epithelization time was noted as the number of days after wounding required for scar to fall off leaving no raw wound behind.

Incision wound model

In the incision wound model, rats depilated by removing hairs at the dorsal thoracic region before wounding. Rats were anaesthetized by diethyl ether prior to incision. Six centimeter long paravertebral incisions were made through full thickness of skin on either side of vertebral column of the rat. The wounds were closed with interrupted sutures of one centimeter apart (14).

The rats are categorized into seven groups (n=6). The animal of group I was treated as control and only ointment base applied topically. The animal of group II, III and IV were treated with ointment of pet ether, chloroform and ethanol leaves extract of *Calendula officinalis*and group V, VI and VII were treated with ointment of pet ether, chloroform and ethanol roots extract of *calendula officinalis*. All the samples were applied topically once daily for 10 days, starting from the day of wounding. The sutures were removed on 8th post wounding day. The tensile strength of wounds was measured on 10th day following continuous water flow technique.

Tensile strength in incision wound model

The tensile strength was calculated in incision wound model. On 10th day the rats were again anesthetized and each rat is placed on a stack of paper towel on the middle of the board. The amount of the towel could be adjusted in such a way so that the wound is on the same level of tips of the arms. The clamps are then carefully clamped on the skin of the opposite side of the skin of wound at a distance of 0.5 cm away from the wound. The longer pieces of the fishing line are placed on the pulley and finally to the polyethylene bottle and the position of the board is adjusted so that the bottle receive a rapid

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and constant rate of water from the large reservoir, until the wound began to open. The amount of water in polyethylene bag is weighted and consider as tensile strength of the wound (15).

3. RESULT & DISCUSSION

The studies on excision wound healing model reveals that all the seven groups showed decreased wound area from day to day. There was noticeable homogeneity in the wound contraction observed for animals in the experimental groups compared with the control group. However, on 16th post wounding day, animals of control group showed 84.53%) of healing (which may be due to self-immunity of the animals) whereas ointment of chloroform and ethanol leaves extract of *Calendula officinalis* treated group (III & IV) showed 96.60% &97.79% healing. On the other hand, ointment of chloroform and ethanol roots extract of *Calendula officinalis* treated group (VI & VII) showed 97.64%& 100% of wound healing. The end scar formed was a fine linear white scar that was visible on the flank of the animals. All readings are found to be statistically significant and comparable with control (Table No. 01 & Figure No. 1)

Table No.01 Percentage wound contraction in excision wound model

	Area of wound closure (sq mm ± S.E.M)				
Groups (n)	**4th day**	**8th day**	**12th day**	**16th day**	**Epithelization period (Days)**
I	228.20±0.92 (52.60%)	168.88±0.98 (64.28%)	120.93±1.08 (76.40%)	68.60±1.12 (84.53%)	24
II	224.17±0.92 (55.16%)	160.88±0.92 (67.82%)	102.93±0.88 (79.41%)	59.61±1.13 (88.07%)	22
III	166.86±1.42* (65.84%)	74.16±1.08* (82.14%)	28.28±0.87* (92.50%)	13.03±0.86* (96.60%)	18
IV	165.76±1.40* (66.84%)	74.36±0.98* (85.12%)	27.33±0.87* (94.53%)	11.03±0.68* (97.79%)	17
V	206.97±1.51* (58.60%)	113.80±0.89* (77.23%)	53.93±1.30* (89.21%)	22.14±0.75* (95.57%)	20
VI	160.80±1.24* (69.94%)	71.16±1.28* (86.45%)	24.18±0.78* (95.60%)	10.12±1.06* (97.64%)	17
VII	150.26±1.20* (68.84%)	40.26±1.28* (88.12%)	10.58±0.85* (94.53%)	00.00±00.00 (100%)	16

Initial wound area approx. 500 sq mm

≈ n = 6 animals in each groups.

≠ Result expressed as Mean Area ± S.E.M.

* P≤ 0.01 indicates significant when compared with control.

Ψ Figure in parenthesis indicate percent wound contraction.

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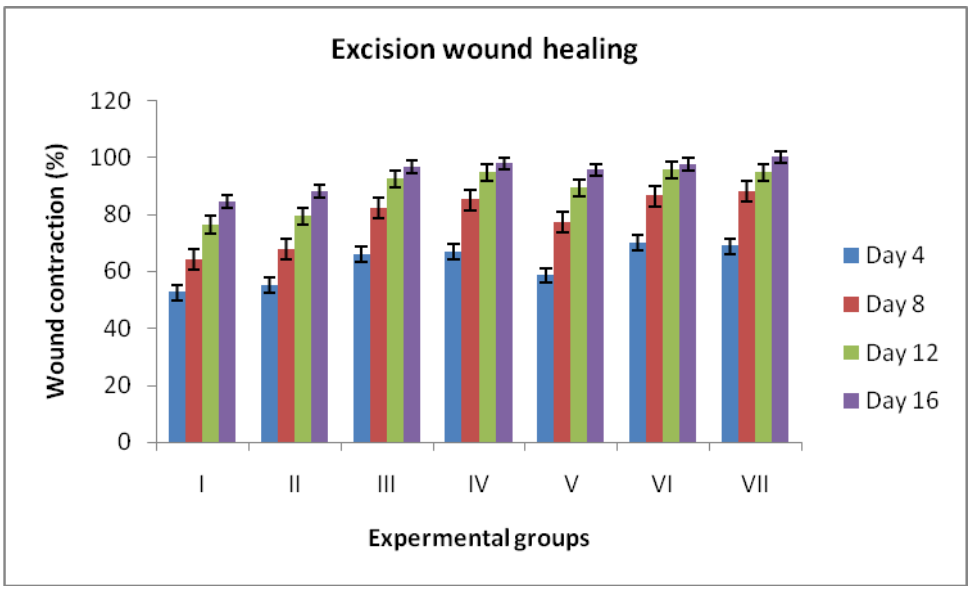

Figure No. 01 Excision wound healing (% wound contraction)

In incision wound model leaves and roots extract's ointment treated group of both *Calendula officinalis* showed better tensile strength when compare with control group. Calendula officinalis ethanol extract's ointment treated group showed better result (Table No. 02 & Figure 02).

Table No. 02 Tensile strength in incision wound model

Groups	Tensile strength (in grams)
I	330.66±1.22
II	332.60±1.04*
III	381.66±1.20*
IV	411.16 ±1.13*
V	360.20±1.28*
VI	398.32±1.08*
VII	420.12 ±1.03*

≠ Result expressed as Mean Area ± S.E.M., * P≤ 0.01 indicates significant when compared with control.

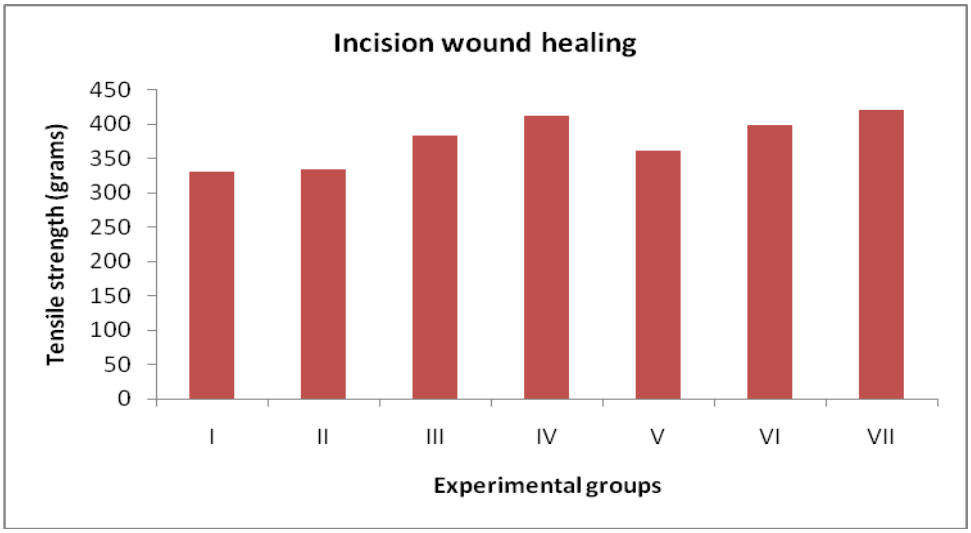

Figure No. 02 Incision wound healing (tensile strength)

4. CONCLUSION

The result of the present study showed that the wound healing activity of calendula officinalis extracts for both excision and incision wound. All types of extracts of both part leaves & roots exerted wound healing activity, but root extracts (pet ether, chloroform, and methanol) of calendula officinalis showing significant activity as compared to all leaves extract.

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