SHORT COMMUNICATION

WOUND HEALING ACTIVITY OF FLOWER EXTRACT OF *CALENDULA OFFICINALIS*

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ABSTRACT

The effects of oral and topical application of *Calendula officinalis* flower extract on excision wounds made in rats were checked. The parameters assessed were the days needed for re-epithelization and percentage of wound closure. The hydroxy proline and hexosamine content in the granuloma tissue of the wound was also measured. The percentage of wound closure was 90.0% in the extract-treated group, whereas the control group showed only 51.1% on the eighth day of wounding ($p < .01$). The days needed for re-epithelization were 17.7 for the control animals; extract treatment at a dose of 20 or 100 mg/kg b.wt reduced the period to 14 and 13 days, respectively. A significant increase was observed in the hydroxy proline and hexosamine content in the extract-treated group compared with the untreated animals. The data indicate potent wound healing activity of *C. officinalis* extract.

KEYWORDS

antioxidant, *Calendula officinalis*, hexosamine, hydroxy proline, wound healing

INTRODUCTION

Wound healing involves a complex series of interactions among different cell types, cytokine mediators, and the extra cellular matrix. The cascade of wound-healing events starts with homeostasis, inflammatory cell recruitment,

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cell proliferation, and remodeling /1/. The inflammatory phase of wound healing is mediated by several factors, including cytokines, prostaglandins, and free radicals that induce the recruitment of white blood cells to the area of wound. As the generation of excessive free radicals will occur, which damages the growing connective tissue, wound healing will be delayed. Similarly when excessive inflammation is present, proteases are no longer selective against the debris as they degrade growth factors, receptor sites on cells, and prevent fibroblasts and other wound-healing cells from their normal functioning. This will again delay the healing process.

Many drugs of plant origin that have been reported to possess potent wound healing activities /2-4/ were found to have free radical scavenging activity. *Calendula officinalis* Linn. (Compositae), which is widely used in homeopathic medicine as anti-inflammatory agent, has been reported to have anti-bacterial /5/, anti-fungal /6/, and anti-viral /7/ activities. Recently we evaluated the antioxidant potential of *C. officinalis* flower extract in vitro and in vivo /8/ and found that the extract possesses superoxide-, hydroxyl-, and nitric oxide radical-scavenging activity. The stable free radicals DPPH and ABTS were also scavenged by *C. officinalis* extract, and lipid peroxidation was inhibited. Treatment with the extract enhanced the anti-oxidant status as seen by the increased level of endogenous antioxidants, catalase superoxide dismutase, and glutathione in animals. In the present study, we report the wound-healing potential of *C. officinalis* flower extract in an animal model.

EXPERIMENTAL

Chemicals and preparation of extract

Glucosamine hydrochloride was purchased from Sisco Research Laboratories, Mumbai. Hydroxyproline was obtained from Sigma Aldrich, USA. Paradimethylaminobenzaldehyde was purchased from E Merck, Mumbai, India. All the other reagents and chemicals used were of analytical reagent grade. Fresh *Calendula* flower tops used for extraction of the active components were collected from Government Botanical Gardens, Ooty, Nilgiris during January 2006 and were authenticated by Dr. T. Subbaraju, J.S.S. college, Ooty; the voucher specimen was deposited at Amala Ayurvedic Research Centre (Voucher No: Co05). Extraction was done as per standard Homeopathic Pharmacopoeia (9). *Calendula* flowers (700 g) were extracted with 450 mL ethyl alcohol by masturation. The material was placed in a wide-mouth bottle and alcohol was added. The jar was stoppered and sealed to prevent evaporation and placed in a dark room at room temperature and shaken daily for two weeks. The resulting clear liquid was decanted and the residue was pressed out through clean linen, which was added to the decanted liquid. The volume was made up to 1 L with alcohol. An aliquot (100 mL) of this tincture of *C. officinalis* was evaporated to dryness in a shaker water bath at 42°C. The yield from 100 mL of extract was 1.1 g. The dried extract was resuspended in known amount of distilled water and used for all experiments.

Animals

Female Wistar rats (150-200 g) were purchased from the Small Animal Breeding Station, Mannuthy, Kerala, India, housed in well-ventilated cages under controlled conditions of light and humidity, and provided with normal mouse chow (Sai Durga Food and Feeds, Bangalore, India) and water *ad libitum.* All animal experiments were conducted as per the instructions prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India, and implemented through the Institutional Animal Ethical Committee of the Research Centre.

Determination of wound-healing activity

An excision wound of 4 cm^2 was made on the shaven back of female adult Wistar rats by removing full thickness skin from that area under light ether anesthesia. The day on which the wound was made was considered day 0 /10/. Four groups of 6 animals each were used, of which group I served as untreated control. Groups II and III received 20 and 100 mg/kg b.wt extract orally for 10 consecutive days. Group IV received a topical application of *Calendula* extract. The wound area was traced on 4^{th}, 8^{th}, 12^{th}, 16^{th}, and 20^{th} day of wounding, the area was calculated graphically, and the percentage of wound closure was calculated. The time taken for epithelization was recorded as indicated by the falling of the scar leaving no raw wound left behind on different days.

TABLE 1

Effect of *C. officinalis* on wound closure on different days after wounding

Group/ dosage	Percentage wound closure					
	Day					
	0	4	8	12	16	20
Control/ none	0	32.4 ± 6.4	51.5 ± 8.9	94.1 ± 1.9	97.3 ± 0.7	99.7 ± 0.1
20 mg/ kg b.wt	0	43.5 ± 4.8**	91.7 ± 2.5**	96.9 ± 0.8**	99.3 ± 0.6**	99.9 ± 0.1**
100 mg/ kg b.wt	0	60.7 ± 3.7**	93.9 ± 2.3**	97.9 ± 1.1**	99.9 ± 0.2**	100 ± 0.0**
Topical application	0	42.2 ± 2.9**	86.9 ± 6.9**	97.1 ± 1.4**	99.7 ± 0.7**	100 ± 0.0**

Values are expressed as percentage of wound closure compared with initial value. **p < .01

TABLE 2

Effect of *C. officinalis* on the period for epithelization after wounding

Group	Days until re-epithelization
Control/none	17.7 ± 0.8
20 mg/kg b.wt	14.0 ± 1.1*
100 mg/kg b.wt	13.0 ± 0.6**
Topical application	16.1 ± 0.4

*p < .05 , ** p < .01

TABLE 3

Effect of *C. officinalis* extract (100 mg/kg/b.wt.) on collagen hydroxyproline and hexosamine content in the granuloma tissue of wounded rats

Parameter measured	Treatment	Day 5	Day 10	Day 15
Collagen hydroxyproline (mg/g dry wt tissue)	Extract	7.60 ± 0.40*	16.47 ± 3.95	9.87 ± 2.36**
Control	None	6.10 ± 0.90	14.70 ± 3.99	4.75 ± 0.70
Hexosamine (mg/100 mg dry wt tissue)	Extract	20.70 ± 1.80***	18.19 ± 2.05*	9.22 ± 1.62*
Control	None	12.80 ± 1.25	15.67 ± 3.99	11.75 ± 2.13

*p < .05 , **p < .01 *p < .05, ***p < .001

Analysis of granuloma tissue

Female Wistar rats (n = 36) were divided in two groups. An excision wound (4 cm^2) was made on the shaven back of all animals. Group I served as untreated control and group II received 100 mg/kg/b.wt extract for 10 days. On the 5th, 10th, and 15th day of wounding, six animals from each group were sacrificed and the granuloma tissue was excised, washed in ice-cold saline, and lyophilized. The lyophilized skin tissue was subjected for estimation of collagen hydroxy proline /11/ and hexosamine /12/ contents.

Statistical analysis

The results are expressed as mean ± S.D. Statistical evaluation of the data was done by one-way ANOVA followed by Dunnet's test (*post-hoc*) using the In Stat 3 software package.

RESULTS

Effect of *C. officinalis* extract on wound healing

The percentage of wound closure with and without treatment is presented in Table 1. On the 4th day, extract treatment produced 43.5% and 60.7% wound closure, and topical application produced 42.2% wound closure, which is significantly higher than the wound closure (32.4%) of the untreated control animals. By 8th day, about 90% of the wound was healed in the extract-treated groups whereas for the control healing was only 51.5%. The period required for epithelization is shown in Table 2.

The hydroxy proline and hexosamine content of granuloma tissue indicates the rate of tissue regeneration. The extract-treated group showed a significant increase in hydroxy proline and hexosamine content during the initial days, which directly indicates that the wound regeneration is significantly faster in the treated group (Table 3).

DISCUSSION

The results of the present study indicate that the extract of *Calendula officinalis* flowers has potent wound healing activity. The ability of the

Calendula extract treatment to promote the wound to heal much faster is attributable to its capability to enhance the synthesis of connective tissue, especially collagen. This view is supported by the increased hydroxyproline content of granuloma tissue directly indicating the rate of collagen synthesis. Hexosamine, the ground substratum for collagen synthesis, has been shown to increase during the early stages of wound healing and to decrease thereafter /13/. The results obtained here showing a significant increase in the level of hexosamine in the drug-treated groups on the initial days can be correlated with the wound healing efficiency of the drug.

Reactive oxygen species liberated by phagocytes invading inflammatory sites are attributed to the tissue damage. Hence, agents capable of scavenging free radicals can protect the injured tissue to a great extent. The process of wound healing is promoted by several natural and plant products, with active principles like flavonoids, triterpenes, alkaloids, tannins, and other biomolecules. The flavonoids promote the wound healing property by producing artificial cross linkage /14/. Flavonoids are also potent anti-oxidants. In animal models, carotenoids, mainly β-carotene, can enhance the wound strength /15/. As inflammation can delay wound repair, anti-inflammatory agents have a significant effect on wound healing. Indeed, triterpenoids isolated from *C. officinalis* has been reported to have potent anti-inflammatory activity /16/; in our earlier experiments, we found that the flower extract of *C. officinalis* can inhibit proinflammatory cytokines. This property could also contribute to the wound healing potential of the extract.

Calendula officinalis flowers contain phytochemicals like carotenoids, coumarins, quercetin, protocatechuic acid, faradiol, oleanolic acid, beta-amyrin calenduladiol, narcissin etc. /17/. The major ingredient is the carotenoids, which include β-carotene, lycopene, flavoxanthin, lutein etc. The antioxidant activity of these compounds may be partially contributing to its wound healing activity. As the *C. officinalis* flower extract is rich in carotenoids and flavonoids, the wound-healing property may be through the enhancement of connective tissue synthesis, increased cross linkage, and the inhibition of collagen degrading enzymes, as well as through free-radical scavenging and inhibition of inflammation due to its antimicrobial activity.

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