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EFFECT OF *ONOPORDON ACANTHIUM* ON FULL-THICKNESS DERMAL WOUND HEALING IN RABBIT

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ABSTRACT

In this study the healing properties of a medicinal plant commonly used in Iran, *Onopordon acanthium* known as cotton thistle was investigated. This plant has been used in the traditional Iranian medicine, as an aid in the treatment of wounds. In order to access which part of this plant contained the principle ingredient(s), hydroalcoholic extract of the roots, stems, leaves and flowers were separately prepared. The extracts were formulated into an eucerin-based ointment and applied once daily to a full thickness excision wound (2x2 cm) inflicted on the mid-dorsal area of White Newzealand rabbit. Measurement of the wound surface area was employed as the parameter by which the effectiveness of each preparation was assessed. The wound areas were traced on transparencies and the areas were quantified by placing the transparency film over graph paper and counting the squares. Compared with other extracts, the results showed that the hydroalcoholic extract from the flowers is the most effective. Dose-response curves showed that 0.2% w/w flowers extract has the optimum concentration which induced the fastest rate of healing, inducing a complete healing in 6 days compared with 17 days with the ointment base alone. The results suggest that this extract is an effective preparation in the treatment of wounds in this model.

Key words: Rabbit, Full excision wound, Wound surface area, Dermal wound healing, *Onopordon acanthium*

INTRODUCTION

Wound healing is a complicated interaction of many factors. This phenomenon has mystified early and modern man. This is evident from archaeological findings which showed that ancient man also had to use a variety of tools to deal with various injuries inflicted on him under hard conditions and during wars (1). The skin is the largest organ in the body. Besides being protective, it has immunological and hormonal functions. The skin has three main layers: the dermis, epidermis, and the hypodermis. The main cells which have been implicated in the contraction and closure of wounds are the myofibroblasts (2). The cells, located in the faecia in the hypodermal layer, have characteristic features of both smooth muscle cells and fibroblasts (3). A feature common with smooth muscles cells is that nuclei unlike to those of fibroblasts, contain many indentations, reminiscent of contractile cells (4). Despite their responsiveness to a variety of pharmacological agents (5,6,7,8) myofibroblasts are unique and do not respond similarly to smooth muscle cells (9). Interests in finding new drugs from medicinal plants in treatment of various illnesses are growing, and recently simplified recommendations has been suggested (10). In these recommendations, the use of crude plant extract, is supported according to toxicity tests on two different species. These new guidelines may facilitate wider, and easily accessible use of medicinal plants, especially in developing countries as an alternative to currently more expensive synthetic chemicals. In this study, the effectiveness of hydroalcoholic extract of *Onopordon acanthium* (family: *Compositae*, subfamily: *Tubuliflorae*) which is also known as cotton thistle was studied. It is a 9-foot plant that grows in many parts of the world (11). It has

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spiny, winged stem of triangular section covered with fine silvery down. The leaf is long narrow, toothed with prickles, green with white down. The flowering parts are magenta-purple, tufted blooms, 5 cm across, borne in the second summer of cultivation. The roots are dark brown taproots, with beige sides and white astringent flesh. Many uses for the plant including the use of the whole plant and its flowers in decoration, the use of the oil from the seeds as a source for cooking and in lighting lamps have been reported. In traditional medicine it has been used in reliefing cancer and skin ulcers (2). A decoction of the root is reported to diminish excessive mucous discharge (10).

MATERIAL AND METHODS

Animals: Male and female White Newzealand rabbits weighing between 1.3 and 1.7 Kg were purchased from Razi Institute of Ahwaz. The animals were maintained at room temperature conditions between 25±4°C, and 12 h light-dark cycle. The animals had free access to animal chew and water.

Wounding Procedure: The lower middle dorsal area of the rabbit was shaved and an area of 2x2 cm close to the vertebral column was cut using blade size 15 and a template piece of galvanized metal plate which was especially designed for this purpose. The area was rendered painless by subcutaneouse injection of a lidocain solution (2ml, 2%) at and around the area under investigation. After induction of localized anesthesia, the excision was directly made by using the designed template and the outer boundaries were traced and cut to the level of the deep subcutaneous area. The excised area was carefully removed, leaving the deep subcutaneous fascia in place. The animals were gently handled and maintained in position on a wooden piece by another member of the group according to the technique of Cross et al (12). In order to ensure the welfare of these animals, the animals were checked on daily basis for any loss in weight, an indication of possible serious effects of wounding on the normal daily habits in performing essential needs, or swelling which was considered an indication of infection at the site of wounding. Non of these signs were observed.

Preparation of the Plant Extract: A sufficient amount of the whole plant was collected from Ahwaz suburban areas, identified by botanist as being of species *O. acanthium*, cleaned from mud and dust with water and allowed to dry under shade. After drying, the roots, stems, flowers and leaves were separated. Each part of the plant was then separately broken down into smaller pieces using manual mortar and then an electrical mortar to produce a fine powder. Each of the plant parts was separately placed in a soxhlet for a total of 9 h (three occasions of 3 h each in a separate fresh 70% hydroalcoholic mixture). The extract was concentrated under vacuum at 75°C. The extract was concentrated up to 6% for the stem to 3% for the flower, to 2% for the leaves and to 2.5% for the root (as measured with aid of a picometer). These solutions were used as stock solutions.

Preparation of the Ointment: Eucerin was used as the ointment base, and depending the concentration of the stock hydroalcoholic extract of each part of the plant, a suitable quantity of each extract was diluted to produce 0.1 and 1% w/w concentration. 100 g of each ointment was prepared.

Experimental Protocol: Following the induction of the wounds, the areas were traced 10 times on a separate transparency for each animal on daily base. The areas were quantified, using a magnifying glass, by placing the transparency film over graph paper and counting the squares. Each of the ointments prepared was applied to a group of 7 rabbits. The results were compared with control given eucerin alone and presented as percentage of reduction in wound surface area employing the general equation (13).

$$\%\ \text{reduction in wound surface area on day } x = 100 - \frac{\text{area of wound surface area on day } x}{\text{area of wound surface area on day } 0} \times 100$$

Preliminary experiments were conducted in order to find out the part of the plant which is most active in the healing process in this model. For this purpose, 0.1 and 1% w/w

ointments of each preparation were tested on 5 groups of seven rabbits. Preliminary experiments showed that there were no significant differences in the rate of wound healing between control air-dried and those treated with eucerin. In order to find out the optimal concentration of the flower extract which causes healing of the wound at fastest rate, ointment preparations of the flower extract at concentrations of 0.025 ,0.05, 0.1 ,0.2 and 0.4% w/w were tested.

Statistical tests: The results were analyzed using analysis of variance (ANOVA) followed by Tukey method of analysis. The level $p<0.05$ was considered significant.

RESULTS

Effect of different parts of the plant: The results from these experiments showed that extract from the flower contained the principle ingredient(s). Extracts from other parts of the plant prepared at the same concentration did not show significant differences from the eucerin control group. The results for 0.1% w/w preparations are shown in Fig. 1. Similar effects were observed with 1% w/w extracts from different parts of the plant (results not shown).

Dose-Response effects of hydroalcoholic extract of the flowering part of O. acanthium: Following the results from the previous section, which showed that the flower extract of the plant contained the principle ingredient(s) and was responsible for the observed pharmacological activities, a dose-response experiment was carried out to find out the most suitable concentration of the extract that induces greatest rate of healing. The same experimental protocol was repeated on six groups of 7 rabbits. For the control group only eucerin was applied. For other groups, hydroalcoholic extract at concentrations of 0.025, 0.05, 0.1, 0.2 and 0.4% w/w in eucerin base were applied daily and the same parameters were measured. The results from this set of experiment showed that 0.2% w/w concentration of the flower extract was the optimum concentration, which reduced the wound surface area. Complete healing was achieved at this concentration by the sixth day compared with 11, 11, 8, 11 and 16 days for 0.025, 0.05, 0.1, 0.4% w/w and control respectively. The time taken to achieve 50% reduction of the wound surface area was extrapolated (Fig. 2). The results showed that 0.2 and 0.1% w/w had the fastest rate of healing, inducing 50% reduction in the mean of the wound surface area on day 3.2 compared with mean of 5, 5, 5 and 7.2 days for 0.025, 0.05, 0.4% w/w and control respectively.

DISCUSSION

Results of this study showed that among extracts from various parts of *O. acanthium,* flowers contain the highest concentration of the principle ingredients. In addition, the rate of healing was optimal with 0.2% w/w concentration of the hydroalcoholic extract of the flowering parts. The investigation of the change in wound surface area has been one of the parameters that many investigators have undertaken since Alexis Carrel's introduction of the concept of the measurement of wound surface area as an index for wound contraction (14). However, there is no universal consensus on the methods used for expressing the results. Despite this, many investigators adopted this parameter as a mean to assess the role of a variety of indigenous substances and to investigate the mechanisms governing the healing process (12, 15). In this study, two methods for comparison of the results were used. In the first method, the statistical means of comparison of the actual wound surface area on daily bases were calculated. In the second method, the time taken for 50% reduction in wound surface area (13) was considered. The results from both methods showed close correlation. The change in wound surface area reported to follow three distinct phases: an initial delay, followed by an exponential rapid phase and finally a plateau consolidation phase (12, 15). The number of the myofibroblasts of wounds has been reported to increase dramatically on the third day and decline on seventh day in the rat (16). The results in the present study clearly show that 0.2% w/w hydroalcoholic extract from the flowers of *O. acanthium* reduced the wound surface area to 50 % by the third day, a highly significant difference from control which produced similar effects on the day of 7 of

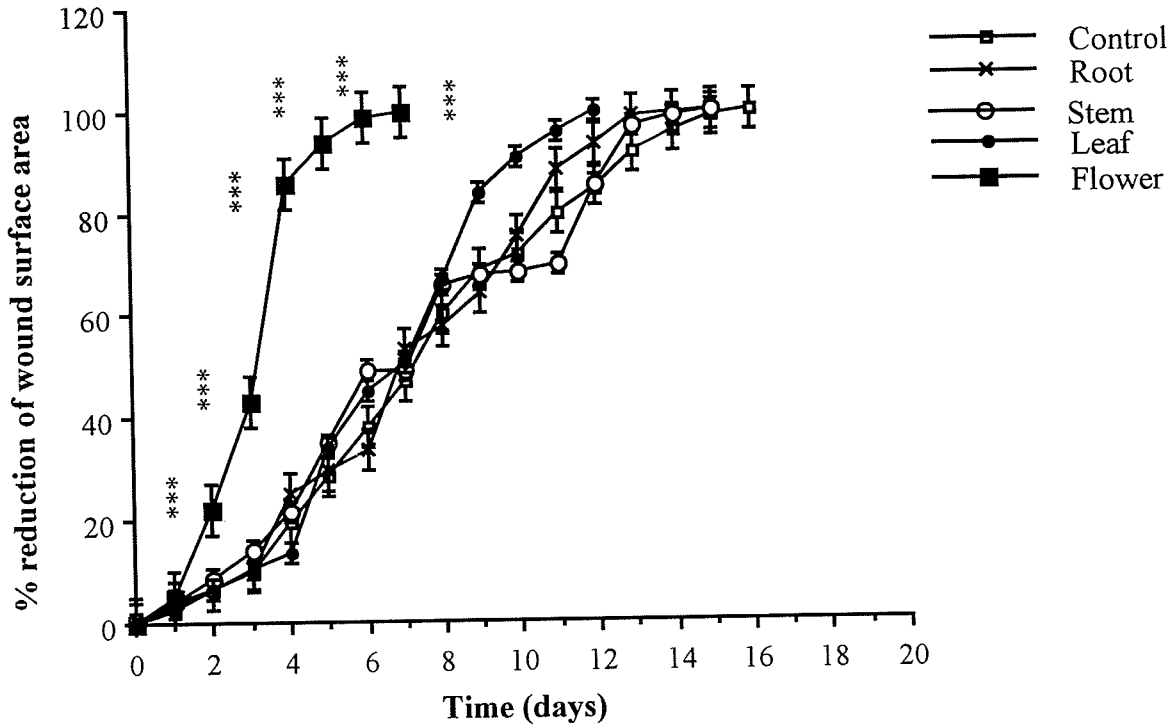

Fig 1. Comparison of the effectiveness of 0.1%w/w hydroalcoholic extracts prepared from root, stem, leaf and flowering parts of *O. acanthium* on the rate of wound healing of a full-excision dermal wound of the rabbit. ***P<0.001 from eucerin-treated control group, ANOVA test followed by Tukey method of analysis, n=7.

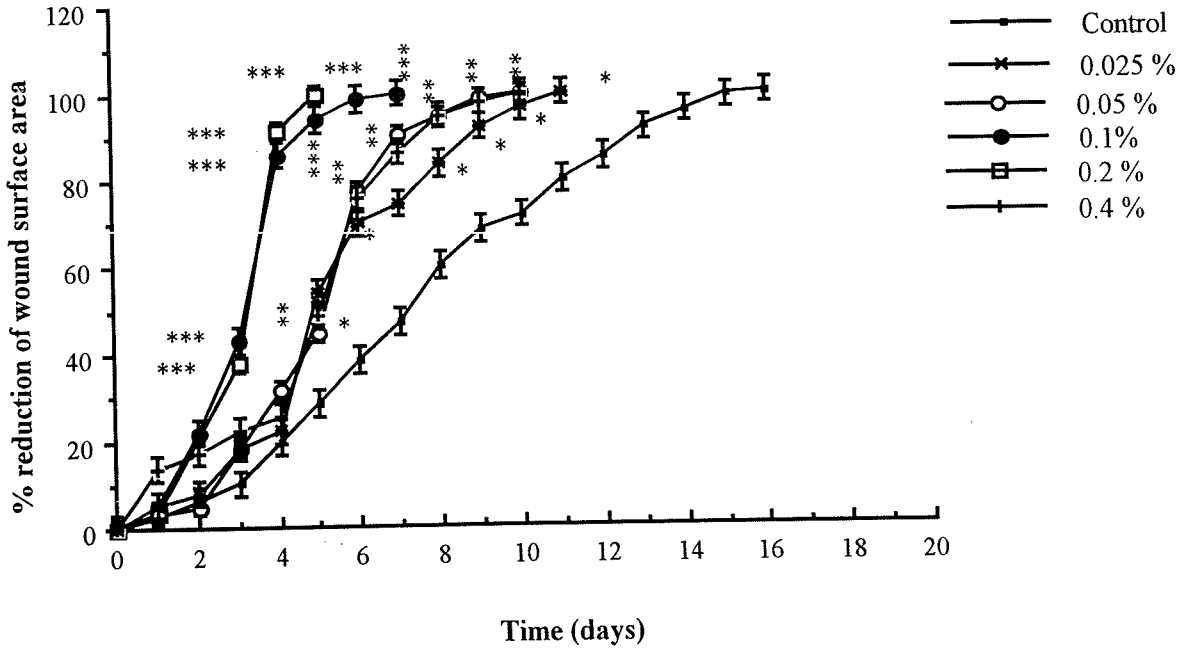

Fig 2. Dose-response relationship of the hydroalcoholic extract from the flowers of *O. acanthium* on the rate of healing on full excision dermal wound in rabbit. Optimum healing rate was observed with 0.2%w/w concentration of the hydroalcoholic extract compared with control. *P<0.05, **P<0.01 and ***P<0.001 from eucerin-treated control group, ANOVA test followed by Tukey method of analysis, n=7.

wounding. This may suggest that this extract may have induced an early increase in the proliferation and/or activity of the myofibroblasts, the principle cells implicated in wound contraction (2,3), in the wound environment. Further work is under way in order to investigate this aspect by histological and histochemical methods. The usefulness of hydroalcoholic extract of the flower parts of *O. acanthium* in speeding up the healing rate compared with other parts of the plant and ointment base alone suggests that the principle ingredient(s) are mainly in the flower part of the plant. In addition, the optimum concentration for this extract was 0.2%w/w, a useful guide both for the ease of preparing and as an indicator for the potency of this preparation as a powerful agent in wound healing.

CONCLUSION

The problems associated with injury and wounding is a universal one and this study has attempted to clarify the role of a medicinal plant in this perplexed field of research. In this study the crude extract of hydroalcoholic extract of the flowers of *O. acanthium* was used as an agent for speeding up the rate of healing of an excisional wound in the rabbit. It is shown that this plant contains compound(s) that may have this potential. It is suggested that this plant may have a role in this process. Further studies are needed to elucidate structure and mechanism(s) of action of its active ingredients.

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