Effect of Achillea millefolium extract in wound healing of rabbit

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Abstract

Objective: To evaluate the effect of hydroalcoholic extract of Achillea millefolium (yarrow) on the wound healing in rabbit. Material and methods: Wound healing effect of yarrow extract in eucerin base at concentrations of 2, 5 and 10% w/w were tested topically on full thickness excision wound in rabbits. Such effect was compared with phenytoin cream (1%) as a standard healing agent. Results: Yarrow extract creams significantly reduced the rate of wound healing compared to no-treatment, eucerin. Five percent yarrow extract cream showed better healing profile, and the rate of healing was significantly (p<0.01) better than other groups. Conclusion: Yarrow showed a considerable potential for wound healing possibly by accelerating the collagenation and proliferation phase of wound healing.

Key words: Achillea millefolium, wound healing, full thickness wound.

1. Introduction

Wound repair is a natural reaction to injury which results in restoration of tissue integrity. Repair process of wound will complete within 3 phases including: inflammation, proliferation and remodeling. There is a similarity between wound healing in human and certain animal species. Therefore, the present study employed a model of rabbit wound healing for the evaluation of Achillea millefolium hydroalcoholic extract.

Numerous drugs with natural (Quince seed mucilage and Ginko biloba) or chemical (e.g. phenytoin, zinc oxide, ketanserin, dexpantenol) origin have been employed to accelerate the rate of wound healing [1 - 4]. Achillea millefolium (Common name: Yarrow) is a plant native to Europe, Asia and north America. In Iran it can be found in north, around the Alborz mountain and in Azerbaijan, Lorestan, Isfahan, and markazi provinces [5]. Yarrow has been used as a traditional medicine by many cultures for hundreds of years. Yarrow tea is used as a diaphoretic remedy to treat fevers and colds in north America and

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United States. In Germany, yarrow flowers is licenced as standard medicinal tea for the treatment of billiary and gastrointestinal disorders [6].

2. Materials and methods

2.1 Plant material

Dried aerial parts of *Achillea millefolium* was purchased from Goldaru Co. Isfahan, Iran. The plant was taxonomically identified at the department of Botany, School of Agriculture, Ahwaz university. Plant was powdered using a grinder. 100g of this powder was placed in a beaker and 1000ml of 70% ethanol was added. The mixture was left in room temperature for 3 days. The extract was separated and remaining plant was extracted by more ethanol after 2 days. The extract was filtered by Wattman (No 10) filter paper and concentrated by vacuum evaporation. The density of concentrated extract was determined. Creams of 2%, 5% and 10% yarrow extract in eucerin were prepared.

2.2 Animals

Newzeland rabbits of either sex weighing 1.8-2.1kg were used during the study. Animals were purchased from Razi institute, Karadje, Iran. Before and after surgery the animals were housed individually in aluminium cages. They were allowed to feed on a standard, commercial pellet diet (Shushtar, Khorakdam Co. Iran) 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 relative humidity of 45% to 55%.

2.3 Wound healing studies

A full thickness wound was made in the skin of the test animals according to the model of Cross *et al* [7] and the experiment was performed according to the modification of Hemmati and Mohammadian [1]. Hairs of lower back and left flank of the test animals were fully shaved and cleared, the desired area was locally anaesthised with the subcutaneous injection of lidocaine (2%), the animal was held in standard crouching position, and the mobile skin of flank was gently stretched and held by the fingers.

A metal template measuring 20x20 mm² was placed on the stretched skin and an outline of the template was traced on the skin using a fine-tipped pen. The wound was made by excising the skin, within the border of the template to the level of loose subcutaneous tissue, using a size 15 scalpel blade and a forceps.

Wounds of animals were treated topically with eucerin containing 1% phenytoin (Darupakhsh Co. Iran) as a standard healing agent, or eucerin containing yarrow extract 0, 2%, 5% and 10%. Wounds of untreated group were not treated with any healing agent. The animals were subsequently returned to cages in the previously described holding room.

Bedding in the cages were changed daily and cages were kept clean to avoid infection of wounds. Wound dressings were done twice daily. Test animals with infected wound were excluded from the study. Only one animal from untreated group had infection and was excluded but substituted later. All ethical issues were considered in the surgical procedure and during the treatments.

In order to quantify the rate of wound healing, each test animal was held in the standard crouching position after every 24 h and outline of the wound was traced on a transparent plastic sheet using a fine-tipped pen. Measurement errors were minimised by repeating each measurement three times and using an average of the measurement in all calculations. The area of the wound on the first day was considered as 100% and the wound areas on subsequent days were compared with the wound on the first day.
Healing percentage was the difference between the initial wound and the healing wound.

2.4 Statistical analysis

It was performed using one way ANOVA followed by multiple comparison with Dunnet’s test. The differences were considered significant when p<0.05

3. Results

Comparison of untreated and eucerin treated animals showed no significant differences in the rate of healing and healing was completed in 21 days in both groups (Fig 1).

In phenytoin-treated group, 17 days was required for complete healing (Fig 1), while yarrow extract creams of 2%, 5% and 10% could accelerate the rate of healing as evidenced by the decreased healing time of 15, 14 and 16 days, respectively (Fig 2).

Yarrow extract cream of 5% produced best rate that was better than phenytoin (1%). Significant (p<0.05) differences between eucerin group and 5% yarrow extract cream was observed from second day of treatment persisted until the end of the study. There was 100% wound healing, achieved after 14 days of treatment with 5% yarrow extract cream. The wound healing effect of 5% yarrow extract cream was more pronounced as compared to 1% phenytoin (Fig 3).

4. Discussion

Healing of wound is a complex phenomenon involving various phases e.g. coagulation, inflammation, collagenation, wound contraction and epithelization. While the phases between coagulation to collagenation are intimately interlinked, the phases of wound contraction and epithelization are independent to each other and run concurrently [8].

In the present study excision wound model for contraction and epithelization was employed. Although numerous studies have been done regarding the pharmacological properties of Achillea millefolium [9], wound healing effect of this plant has not been documented very well. Our results undoubtedly indicates that the extract of yarrow is able to accelerate the rate of wound healing.

![Figure 1](image1.png)

Figure 1. Comparison of the wound healing in no-treatment, eucerin and phenytoin treated groups (n=5); values expressed as mean±SEM; *p<0.05 vs no treatment.

![Figure 2](image2.png)

Figure 2. Comparison of the wound healing in no-treatment, eucerin and 2%, 5%, 10% yarrow extracts treated groups (n=5); values expressed as mean±SEM.
This conclusion comes from the faster contraction of wounds treated with yarrow extract compared to control or untreated wound. Yarrow extract was more potent than phenytoin which is commercially available for clinical uses. The course of healing by 5% yarrow extract was 14 days which is 3 days shorter than 1% phenytoin. The percentage of healing with yarrow extract was significantly higher than eucerin from the second day of treatment and continued until the completion of healing.

The mechanism of yarrow extract in wound healing cannot be explained from the present study and it merits further detailed investigations for its clinical evaluation and the details of mechanism of action.

However, significant differences of yarrow extract treated groups suggesting that yarrow extract may be effective in proliferation and remodeling phase. Perhaps it is able to stimulate the myofibroblasts contraction for the faster closure of wound. Active ingredients of yarrow such as achillein, apigenin, amino and fatty acids [10] may contribute to the healing effects of yarrow extract.

Presence of hydolysable tannins in yarrow extract [11] may cause coagulation of surface proteins and prevention of wound infection and assist the wound for faster healing. However more studies are required to elucidate the exact mechanism of yarrow extract in wound healing.

References
