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Experimental Evaluation of Repair Process of Burn Wound Treated with Aqueous Extract of *Achillea millefolium* on Animal Model: Clinical and Histopathological Study

[1]F.S. Sabet Jalali, [2]Hossien Tajik and [3]Aliasghar Tehrani
[1]Department of Clinical Sciences, [2]Department of Food Hygiene, [3]Department of Pathobiology,
College of Veterinary Medicine, P.O. Box 57155/1177, Urmia University, Urmia, Iran

Abstract: This study was performed to evaluate the accelerating effect of aqueous extract of yarrow on the burn wound healing in an animal model. Ten male adult white Dutch rabbits, with mean weight of 2,000±250 g were studied. Burn wounds were created in dorsal region of each animal, according to Hoekstra model. The experiment wounds were treated with 5 mL of aqueous extracts of yarrow every day. The control wounds were only washed with the same amount of normal saline. The wounds were photographed and compared for rate of wound contraction with digital scanning software. Specimens were taken for histopathological examinations on day 21. Significant differences were seen between the experiment and control wounds for the rate of contraction. On histopathological evaluation granulation and epithelization were more pronounced and the collagen fibers were also more orderly arranged in the experiment group. It is concluded that topical application of aqueous yarrow extract, as used in this study could improve rate of burn wound healing in the rabbit.

Key words: Yarrow, aqueous extract, burn wound, rabbit, experimental evaluation, repair process

INTRODUCTION

Medicinal herbs have been used for thousands years to heal wounds, skin ulcers, pressure sores, bed sores and burns. Ancient wound healing recipes suggest a number of herbs, herbal extracts and oils, creams and ointments with alleged wound healing properties (Arora and Kaur, 1999). One of these medicinal plants is yarrow (*Achillea millefolium*), this herb has a high reputation and is widely employed in herbal medicine, administered both internally and externally (Moerman, 1998). *Achillea millefolium*, belonging to the Asteraceae family, is used widely in many parts of the world (Chevallir, 1996).

Yarrow seems to have originated in European folk medicine. From the center of origin, this herb has been spread east (Perry, 1980). *Achillea millefolium* has been used in Europe since at least the time of Dioscorides. As a doctor with the Roman army, he used it for healing wounds, an important use for soldiers; hence the old name *wound wort*. He wrote 'pound the leaves and put them on a fresh wound to close and heal it, to clear heat and inflammation from the wound and to congeal the blood (Kreutterbuch and Dioscorides, 1960).

For many centuries, various species of genus Achillea have been used as folk medicines for the curing of various diseases. Yarrow now has one of the widest ranges of applications of any herb used in the west. It is used for disorders of the respiratory, digestive, hepatobiliary, cardiovascular, urinary and reproductive systems (Hoffmann, 1999; Bradley, 1992; Mills, 1994; Bown, 1995; Blumenthal *et al*., 1998).

For the broad spectrum usage of *Achillea millefolium* and their possible medical applications have attracted the attention of plant physiologists and chemists (Saeidnia *et al*., 2005).

Extracts of *A. millefolium* have demonstrated antimicrobial activity against a wide range of bacteria (Orzechowski and Pharmazic, 1972).

Studies were carried out on the antioxidant and antimicrobial properties of yarrow and its derivatives such as essential oil and water-soluble compounds (Barel *et al*., 1991). Linalool, found at up to 26% of the essential oil fraction in hexaploids, which are the most common subspecies of *A. millefolium*, has been shown to inhibit 17 types of bacteria and 10 fungi (Pattnaik *et al*., 1997).

It also contains the anti-inflammatory agent azulene, though the content of this varies even between plants in the same habitat (Bown, 1995).

In spite of yarrow widespread health use throughout history and around the world and the appreciated belief that it helps in maintaining good health, warding off

Corresponding Author: Farnood Shokouhi Sabet Jalali, Department of Clinical Sciences, College of Veterinary Medicine, P.O. Box 57155-1177, Urmia University, Urmia, Iran

illnesses, however, no research has yet been reported on the effects of this herb on experimental burn wound healing. Therefore, the objective of this research was to evaluate *Achillea millefolium* in form of aqueous extract for promotion of burn wound healing in the rabbit as an animal model.

MATERIALS AND METHODS

Plant material: Aerial parts of *A. millefolium* were collected in July 2006, during the flowering period and the vegetative phase, from Urmia area in west north of Iran. Taxonomic identity of the plant was confirmed by comparing collected voucher specimen with those of known identity in the herbarium of the Department of Botanical Sciences, Investigation Institute of Agriculture Organization of Iran. Aqueous extracts were also prepared by macerating 100 g of dried and finely ground aerial parts in 1 L absolute distilled water overnight, respectively. Aqueous dried residues were obtained by freeze-drying. Water extracts of *A. millefolium* were sterilized by filtration. The preparation of *A. millefolium* extract used was based on Aburjai and Hudaib (2006).

Animals: In this investigation, we studied 10 male white Dutch rabbits, weighing a mean of 2,000±250 g standard deviation, all 4-6 months old. The rabbits were obtained from the Experimental Animal Laboratory, Urmia University, Urmia. The animals were randomly divided into control (n = 5) and experimental (n = 5) groups.

Rabbits were housed under standard laboratory conditions (12 h light, 12 h dark cycles, with lights on at 8:00 am; 23°C) and maintained on standard laboratory food and water ad libitum. The experimental protocol was also approved by the Animal Ethics Committee of the university. The model of the burn wound was produced according to Hoekstra standard (Brans *et al*., 1994).

On day zero, each rabbit was anesthetized by 50 mg kg^{-1} ketamine hydrochloride, intramuscularly injected, along with 5 mg kg^{-1} diazepam. Animals were positioned in ventral recumbency and hair just behind the shoulders was shaved from the backs then, skin was prepared for aseptic surgery.

Experiment protocol: All animal were subjected to the rectangular burnwounds (20×25 mm^2) using a hot (180°C) brass brick weighing 300 g which was pressed against the shaved skin for 10 sec.

In the treatment group, as a daily procedure, wounds were washed with normal saline. Subsequently, 5 mL of aqueous extracts of *A. millefolium* was applied in a thin layer to the wounds. In the control group, the wounds were only washed with the same amount of normal saline. All the wounds were bandaged with a nonadhesive dressing, which was held in place with an elastic wrap. No antibiotic was used as a pre- or post-operative prophylaxis.

Assessment: The clinical assessment was done throughout the duration of the study and covered the general health conditions as well as the reaction to environment. The process of burn wound healing was especially carefully assessed. The appearance and processes taking place in the tissue surrounding the wounds were evaluated as well as the granulation tissue formation and the process of scar formation. The wounds were photographed on days 0, 7, 14 and 21. All the photographs were scanned and wound areas were measured using digital scanning software (Sigma Scans Pro 5.0, SPSS Science, Chicago, IL).

Variable analyzed included the rates of wound contraction (percent decrease of wound area) (wound area on day 0 minus wound area on day n, divided by the wound area on day 0 expressed as a percentage). The data retrieved from experiment wounds were compared with the control using Student-t-test. $p<0.05$ was considered significant.

The histopathological assessment of the healing process was done on the 21st day of the study. Before material samples were taken for the histopathological tests, animals were subjected to general anesthesia.

The samples were collected from the wounds borders as well as from the middle of the wounds area. Paraffin sections were stained with the standard haematoxylin-eosin method.

RESULTS

The clinical evaluation showed visible differences in the process of wound healing after applying the above mentioned medications. The rates of wound contraction (percent decrease of wound area) in experiment and control wounds are shown in Table 1. In the first day, the clinical pictures of the two groups were similar. Along the necrotic changes, there were inflammatory reactions with a large amount of exudate and a skin oedema around the wound.

Table 1: Comparison of wound area (mm^2)(mean±SD) and percent decrease in the experiment (n=5 rabbits) and control wounds (n=5 rabbits)

	Day			
Group	0	7	14	21
Experiment	524.2±62.24	284.4±82.22* (45.75%)	51.64±40.42* (90.15%)	6.42±4.66* (98.78%)
Control	542.4±41.24	366.2±42.44 (32.49%)	152.26±82.24 (71.93%)	98.16±46.24 (81.91%)

*Significant difference ($p<0.05$)

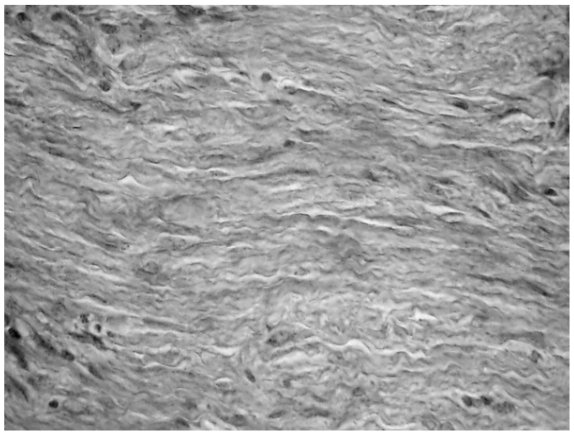

Fig. 1: The 21st day after burn execution, photo-micrograph of the yarrow –treated wound showed more advanced maturation of granulation tissue with dominance of collagen fibers (Area 400x, HE)

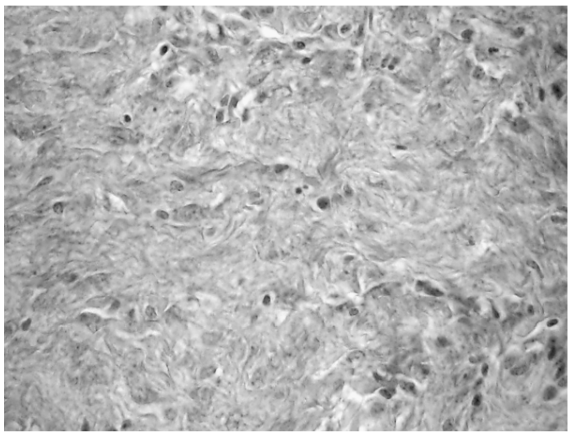

Fig. 2: The 21st day after burn execution, photo-micrograph of the control wound showed loose and irregular granulation tissue (Area 400x, HE)

In the cases treated whit aqueous extracts of *A. millefolium*, the differences in wound appearance in compare to control were visible after 7th day. Oedema and reddening at the skin around of wounds were smaller in experimental group. In the same time, control wounds were only covered with thin, poorly-formed scab.

On the 14th day , in yarrow-treated wounds surfaces became visually smaller than control's wounds. The scabs started to come off at the boundaries of the wound surface and a light-pink scar was visible underneath. This suggested an advanced epidermalization process.

On the 21st day, the wounds were covered with an organized scar and almost healed and the wound gap completely closed. In control group, wound cavity only filled with fresh granulation tissue and wound granulation process on the edges.

During the time of the experiment the animal did not show any signs of excitation and did not react defensively.

From histological aspect, on the 21st day, in yarrow-treated group, the skin defect filled completely with compact granulation and considerable amount of collagen fiber with least amount of inflammatory cells (Fig. 1).

In the same time, in control group, wound cavity filled with fresh granulation (Fig. 2).

DISCUSSION

Today, there is a public interest to usage of medicinal plants in a part of natural and traditional therapies. Yarrow is one of the commonest use of these medicinal plants from the Compositae (Asteraceae) family. There are many reports about therapeutic application of yarrow in treatment of various diseases (Hausbn *et al*., 1991; Moore, 1993; Singh and Blumenthal, 1998; Taylor and Francis, 2001; Dalsenter *et al*., 2004; Lemmens *et al*., 2006; Lans *et al*., 2007). But there are few researches on the accelerating effect of *A. millefolium* on wound healing (Bradley, 1992; Cavalcanti *et al*., 2006).

The aim of this study was to clarifying the advantageous effects of the aqueous extract of yarrow in treatment of the experimental burn wounds.

According to the results of this study, wound contraction in all groups (control and experiment) occurred at during days 7 through 21, which indicates that the percentage change in wound area was constant during this period. These findings suggest that application of *A.* millefolium extract on full-thickness burn wound significantly increased both the rate and amount of wound contraction.

Table 1 showed that the decrease in wound area was significantly greater at day 21 in the experiment wounds (98.78%) compared to the control (81.91%) ($p<0.05$). The results of this study parallel those of studies involving the application of yarrow in wound contraction in the literature (Nedelec *et al*., 2000; Richey *et al*., 1989). In each of these studies, yarrow was effective at its lowest dose and only a single application was needed to provide a significant effect. Although these studies were taken on incisional wound, but the present study was carried out on burn wound.

Wound contraction involves a complex set of extracellular and cellular interactions that result in the closure of an open wound. Although there are several theories as to the exact mechanism, it is believed that locomotion of proliferating fibroblasts into the newly open wound contributes to the early stages of wound closure (Nedelec *et al*., 2000).

Likewise, after this early stage of dynamic wound contraction, myofibroblasts predominate within the wound and augment early wound closure, as well as aid in remodeling of the recently contracted wound (Richey *et al*., 1989).

Clearly, wound contraction is a fibroblast-driven event that is acutely sensitive to fluctuations in the fibroblast-dependent cellular and extracellular matrix. Although vital to wound closure, wound contraction and shrinkage of the healed scar can lead to scar contractures, which may cause significant functional and cosmetic morbidity.

The histopathological study revealed that pathological process in all of the groups (control and experiment) was similar; the time from the moment the thermal wound was made to the beginning of repair processes in the wound was different depending on the applied remedy.

In the case of control group it was the 14th day, but after applying the aqueous extract of yarrow the repairing process was observed at the 14th day. Epitheliazation of the wound area was accelerated by the topical treatment with yarrow, the epithelial thickness was uniform and normal compared with the control wounds. The collagen accumulation in both sets of wounds was equal, but in the experimental wounds was more orderly arranged.

Potential limitations of this study are the failure to examine long-term effects on the wounds after day 21 and the lack of histologic assessment of the wound bed at different markers in the wound healing process. Specifically, it is not known from the results of this study whether wound contracture continues after 21 days, or if yarrow application only accelerates, but does not increase, the process of wound contracture. Underlying histological change in the fibroblast and collagen components of the wounds as a function of yarrow application is also unknown. Accordingly, future studies should examine the course of wound healing with yarrow treatment for longer periods and should incorporate histological examination of tissue as a measurement variable.

Post-burn contracture, a lower incidence of hypertrophic scar, relief of pain, low cost and easy availability make yarrow extract an ideal dressing in the treatment of burn.

Although there is no clinical evidence of toxicity in topically applied yarrow extract, but *A. millefolium* is known, however, to be an allergenic agent when topically administered.

Some caution should be exercised in the use of this herb since large or frequent doses taken over a long period may be potentially harmful, causing allergic rashes and making the skin more sensitive to sunlight (Foster and Duke, 1990).

Therefore, before topical application of the aqueous extract of yarrow is used clinically in patients for scar tissue formation and contraction of burn wound, the safety of using yarrow for skin lesions must be defined.

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