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Influence of Aqueous Extract of Yarrow on Healing Process of Experimental Burn Wound in Rabbit: Clinical and Microbiological Study

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Abstract: *Achillea millefolium*, belonging to the Asteraceae family, is used widely in many parts of the world. There are many reports about therapeutic application of yarrow in treatment of various diseases. But the documented information about the enhancing effect of yarrow extract on healing of the burn wound is few. The aim of this investigation was a clinical and microbiological evaluation of the accelerating effect of the yarrow (aqueous extract) on the burn wound healing in rabbit. A total of 10 male white rabbits, with mean weight of 2,000±250 g were studied. Based on Hoekstra standard model, burn wounds (20×25 mm^2) were created in dorsal region of each animal. Five mililiter of aqueous extract of yarrow used on the experiment wounds every day for 21 days. In the control group, the wounds were washed with normal saline at same frequency and time of day. Clinical and microbiological examinations of the burn wounds were carried out on 0, 7, 14 and 21 days of the experiment. The wounds were photographed and compared for rate of wound contraction with digital scanning software. Wounds treated with yarrow extract had more improved healing appearance and the rate of contraction in contrast to the control wounds. Furthermore, a lesser total count of microorganisms was found ($2\pm0.4 \times10^4$ cm^{-2}) when yarrow extract was applied on burn wounds compared to the control wounds ($1\pm0.5\times10^5$ cm^{-2}) on the day 21 ($p<0.05$). According to the results of this study, topical application of the aqueous extract of *Achillea millefolium* could enhance burn wound healing process in the rabbit from clinical and microbiological aspects.

Key words: Yarrow, aqueous extract, burn wound, microbiology, rabbit

INTRODUCTION

Today technological progress leads to the increase in the exposure of people to the formation of burns and, at the same time, to the intensification of research on new efficient methods for the treatment of these ailments (Subrahmanyam *et al*., 2001).

Wide burns are an infection gate for many microbes and arising necrosis of tissues is convenient environment for their multiplication. Burns become infected because the wound area is an ideal medium for the multiplication of the infecting organism (Subrahmanyam, 1998).

Number and types of microbes existing in after-burn wounds are significant for the running of biological processes of which final effect should be wound healing (Grzybowski, 2001).

Nowadays, the treatment of burns relies, among other things, on surgical removal of necrotic tissues, supplementation of liquids and wound protection from infection with microbes (Mayhall *et al*., 1983; Molan, 2001).

Preventive local use of antibiotics as a routine therapy does not always ensure the sterility of the wound. This is caused by the inactivation of some antibiotics or development of resistance of microbes (Husain *et al*., 1989; Ivanowska *et al*., 1995).

While current approach to burn injury management have improved patient prognosis, increased morbidity and mortality still remains a major challenge for clinicians (Mason *et al*., 1986).

Taking into consideration the above mentioned conditions, research on new efficient herbal or aromatherapy preparations appear to be done on purpose. Among present conventional methods of treatment, more and more attention is brought to the pharmacologically active fractions received from medicinal plants showing healing properties.

Medicinal herbs have been used for thousands years to heal wounds, skin ulcers, pressure sores, bed sores and burns (Perry, 1980).

Achillea millefolium is one of the ancient used of these medicinal plants.

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According to a legend, Achilles (A hero of Homer Iliad) applied herbs, in particular Yarrow, to heal battle wounds of his fellow warriors (Perry, 1980).

Lonicerus recommended Achillea for clearing putrid matter from ulcers and wounds (Lonicerus, 1962).

Now yarrow 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, 1990; Bradley, 1992; Mills, 1994; Bown, 1995; Blumenthal *et al*., 1998; Barel *et al*., 1991; Pattnaik *et al*., 1997; Aljancic *et al*., 1999).

Despite this fact, the evidence-based information on the accelerating effect of yarrow extract on burn wound healing is few (Bradley, 1992).

In view of the difficulty in the treatment of burn wounds, our goal in this study was to define the inhibitory efficiency of yarrow extract in relation to bacterial contamination of the experimental burn wounds in rabbits.

MATERIALS AND METHODS

Plant material: Aerial parts of yarrow were collected 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 *Achillea millefolium* were sterilized by filtration.The preparation of yarrow extract used was based on Aburjai and Hudaib (Aburjai and Hudaib, 2006).

Animals: In this investigation, we studied 10 male white 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).

Fig. 1: A photograph of experimental burn wound based on standard model at the dorsal region of rabbit

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 burn wounds (20×25 mm^2) using a hot (180°C) brass brick weighing 300 g which was pressed against the shaved skin for 10 sec (Fig. 1).

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. The process of burn wound healing was especially carefully assessed.

The wounds were photographed and all the photographs were scanned and wound areas were measured using digital scanning software (Sigma Scans Pro 5.0, SPSS Science, Chicago, IL). Time elapsed for wound healing was considered in both groups. The rates of wound contraction were analyzed.

Clinical and microbiological [quantitative (total plate count) and qualitative (using specialized microbial medias] examinations of the burn wounds were carried out on 0, 7, 14 and 21 days of the experiment.

The results of total bacterial count and rates of wound contraction were analyzed with a non paired Student's t test. Differences were considered significant if $p<0.05$ (SigmaStat for Windows, version 2.03, Jandel Corporation, San Rafel, CA).

RESULTS AND DISCUSSION

The results of total bacterial count in control and experimental wounds were comparatively shown in Fig. 2.

The total numbers of the strains on the skin were $4\pm0.8\times10^4$ cm^{-2} in control group and $5\pm0.2\times10^4$ cm^{-2} in the experiment group during the microbiological examination of the skin before use of the preparations. At the same time, the growth of *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes* and *Candida albicans* strains were observed.

In the yarrow treated group, the numbers of microbes were $5\pm0.4\times10^4$ cm^{-2} in the first 24 h of the experiment. On the 7th day of the experiment this count was $2\pm0.4\times10^5$ cm^{-2}, however, on day 14 it decreased to the value of $1\pm0.6\times10^5$ cm^{-2}. This value decreased significantly on the 21st day and reached to the $2\pm0.4\times10^4$ cm^{-2}.

At this time, just *Staph. aureus* was isolated from yarrow treated burn wounds.

In the control group, the number of microbes existing on the wound in the first day, were $4\pm0.4\times10^4$ cm^{-2} and gradually increased during the following days to the value of $3\pm0.2\times10^5$ cm^{-2} on day 7. In the 14th day, total numbers of micro-organisms were $4\pm0.6\times10^5$ cm^{-2}. On day 21, the number of microbes to decrease until reached to $1\pm0.5\times10^5$ cm^{-2}. *Strep. pyogenes* was only microorganisms isolated at the end of experiment in this group.

From clinical aspect, burn wounds were healed without any complications in both groups. The rates of wound contraction (percent decrease of wound area) in experiment and control wounds are shown in Table 1.

Today the usage of yarrow extract as a naturopathic remedy appears to be widespread. 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).

While, the documented information about the enhancing effect of yarrow extract on healing of the burn wound is few (Bradley, 1992; Cavalcanti *et al*., 2006). Researches about burn injuries show that the most frequent reason (over 50%) of death is infection (Subrahmanyam *et al*., 2001; Artz and Reiss, 1975). With attention to many researches were shown that extracts

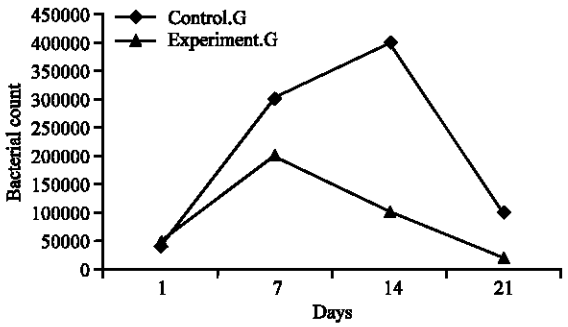

Fig. 2: Result of microbiological examination of burn wounds in experimental and control groups

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 ($p<0.05$)

of *Achillea millefolium* have inhibitory effect on various wound-pathogen microorganisms (Barel *et al*., 1991; Pattnaik *et al*., 1997; Hausbn *et al*., 1991). Therefore, as a hypothesis, it may be considered that yarrow may be poses beneficial and accelerating effect on burn wound healing.

The aim of the present investigation was assessment of efficacy of yarrow extract on microbial contamination of burn wound surface. According to opinion of Barel *et al*. (1991) inhibitory effect of *A. millefolium* has broad-spectrum range on various bacteria and Candida albicans (Barel *et al*., 1991).

This antibacterial effect thought to be related to special bioactive component in essential oil and extract of this plant.

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). An investigation of extracts of yarrow revealed the presence of 5 unsturated hitherto unknown guaianolides with peroxidal property. These components also have inhibitory effect on some microorganisms (Hausbn *et al*., 1991).

Furthermore, *A. millifolium* has been reported to contain some flavonoids with antibacterial efficacy such as rutin and glucosides of apigenin, luteolin and acacetin (Aljancic *et al*., 1999). On the basis of the results of this investigation, on the 21st day, the total number of isolated microorganisms in yarrow treated wounds was significantly less than control group ($p<0.05$).

At the same time, clinical evaluation showed that the decrease in wound area was significantly greater from day 7 and after that in the experiment wounds compared to the control ($p<0.05$). Moreover, wound contraction was gradually increased in both groups; the rate of contraction was clearly greater in the experiment wounds and these wounds approximately complete closed (98.78%) at the 21 day.

These findings are similar to those of studies involving the application of yarrow in wound contraction in the literature (Nedelec *et al*., 2000; Richey *et al*., 1989). Although these mentioned studies were taken on incisional wound, but the present study was carried out on burn wound.

In view of the fact that the present study is very likely the first experimental investigation on the yarrow as a promoter of burn wound healing, our results are not comparable with those of previous works. On the basis of these explanations, topical application of yarrow extract causes significant development in the rate of wound contraction and decrease microbial contamination of burn wound surface.

CONCLUSION

To summarize the evidence, our results suggest that aqueous yarrow extract may be used to enhance the process of wound healing in the rabbit. A.millefolium is known, however, to be an allergenic agent when topically administered (Foster and Duke, 1990). Fortunately, until this time, there is no clinical evidence of toxicity in topically applied yarrow extract. But some caution should be exercised in the use of this herb especially in large or frequent doses. It seems further studies are required to clarify other possible mechanisms leading to the effects of yarrow extract on burn wound healing.

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