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The Efficacy of Alcoholic Extract of Garlic on the Healing Process of Experimental Burn Wound in the Rabbit

[1]Farnood Shokouhi Sabet Jalali, [2]Hossein Tajik, [1]Shahram Javadi,
[2]Bahador Haji Mohammadi and [2]Saeed Shams Addin Athari
[1]Department of Clinical Sciences, [2]Department of Food Hygiene and Quality Control,
College of Veterinary Medicine, Urmia University, P.O. Box 57155/1177, Urmia, Iran

Abstract: This investigation was evaluation of efficacy of alcoholic extract of garlic on the healing of burn wound in rabbit. In this study, 10 male white rabbits, weighing a mean of 2.200±240 g were used. Based on Hoekstra standard model, a rectangular burn wounds (2×2.5 cm^2) were created on back region of each rabbit. The wounds in experimental group were treated with alcoholic extract of garlic (5 mL) every day for 21 days. In the control group, the wounds were washed with normal saline (5 mL) at same frequency and time of day. The wounds were evaluated form clinical (rate of wound contraction) and microbiological (total count) aspects. The clinical findings of the present study were showed wound surfaces in the experiment group were significantly decreased in comparison to the control wounds ($p<0.05$). Microbiological evaluation was showed that total bacterial count of wound surfaces in garlic-treated group was lesser than this variable in the control wounds ($p<0.05$). With attention to these results, alcoholic extract of garlic can be considered as a topical agent for accelerating and enhancement burn wound healing in rabbit.

Key words: Garlic, alcoholic extract, burn wound, microbiology, rabbit

INTRODUCTION

Allium is the largest and most important genus the alliacea family and comprises 450 species, widely distributed in the northern hemisphere. Among them, Garlic (*Allium sativum* L.), is well known specie being used in traditional medicine and food in many countries (Baruchin *et al*., 2001; Sharma and Prasad, 2001; Baghalian *et al*., 2005; Haciseferogullari *et al*., 2005; Lanzotti, 2006). It has long been revered for its therapeutic properties as evidenced by ancient writings from Persia, Egypt, Greece, China and India extolling its merits (Fenwick and Hanley, 1985; Milner, 1996, 1999; Orekhov and Grunwald, 1997; Donkers *et al*., 1999).

Louis Pasteur was reputed to be the first to demonstrate the antibacterial effects of garlic and onion extracts (Sivam, 2001). In recent years, it has been showed that garlic may influence in prevention of the risk of heart disease and cancer (Milner, 1996, 1999; Orekhov and Grunwald, 1997; Yoshida *et al*., 1999).

The most of its therapeutic effects are attributed to specific oil and water-soluble organosulfur compounds, which are responsible for the typical odor and flavor of garlic (Sivam, 2001). The thiosulfinate structure appears to be essential for the bactericidal, antifungal and antiprotozoal properties of garlic, likely reacting with SH-containing enzymes of these pathogens (Reuter *et al*., 1996).

Early steps involved in identifying the active constituents of garlic were the discovery that the compound allicin (allyl 2-propene thiosulfinate) is formed when garlic cloves are crushed (Cavallito and Bailey, 1944; Cavallito *et al*., 1945) and that its formation depends upon the action of the enzyme alliinase of the bundle sheath cells upon the alliin of mesophyll cells (Stoll and Seebeck, 1948).

It has been claimed the antibacterial effect of allicin is broad spectrum (Tsao and Yin, 2001). One problem with the practical use of allicin as a commercial antimicrobial agent is its instability. Its rate of deterioration increases with increasing temperature. In addition to allicin, other products of allicin such as Diallyl Sulfide (DAS), Diallyl Disulfide (DADS) and Diallyl Trisulfide (DATS) and ajoene, which are more stable than allicin have been found to have antimicrobial and antifungal properties (Harris *et al*., 2001). Various bacterial strains resistant to antibiotics such as methicillin resistant *S. aureus* as well as other multi-drug-resistant enterotoxicogenic

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

strains of *Escherichia coli*, Enterococcus, *Shigella dysenteriae*, *S. flexneni* and *S. sonnei* cells were all found to be sensitive to allicin (Chowdhury *et al*., 1991; Gonzalez-Fandos *et al*., 1994; Shadkchan *et al*., 2004). Other bioactive substances such as some proteins, saponins and phenolic compounds can also contribute to therapeutic activity of garlic (Griffiths *et al*., 2002).

Because bioactive component of garlic are easy to obtain or prepare, the medical properties of garlic constituents have been the focus of many studies (Griffiths *et al*., 2002; Bakri and Douglas, 2005; Cooper and Pinto, 2005). Due to the great antimicrobial activity that garlic and onion possess, both vegetables could be used as natural preservatives, to control the microbial growth (Pszczola, 2002).

Although, the topical use of garlic as a naturopathic remedy appears to be widespread for treatment of various diseases, evidence-based information on the boosting effect of garlic extract on wound healing is lacking.

This study is clinical and microbiological evaluations of efficacy of topical application of alcoholic extract of garlic in treatment of experimental burn wound healing.

MATERIALS AND METHODS

Plant material: The garlic was used in the present study, was purchased from local vegetable markets (Urmia, Iran). The garlic bulbs were peeled and ground to form a paste in 100 g quantity. The paste was then dissolved in 1000 mL ethanol in a sterile tube. The solution was then shaking at 24 h at room temperature. Then the content was filtered by filtering paper and Bochner funnel. Finally, the alcoholic extract was extracted by an extractor (Labortata 4000 ECO, Heidolph Co, Germany). The concentration of garlic extract used was based on Aburjai and Hudaib (2006). For sterilization, the extract was filtered by sterilizing filter (Chromafil, 0.22 μ) then sterile prepared extract was stored in 4°C in a refrigerator.

Animals: In this investigation, we used 10 male white rabbits, weighing a mean of 2.200±240 g. 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.

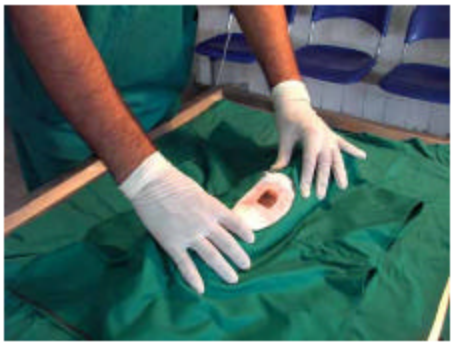

Fig. 1: Experimental burn wound based on standard model at the dorsal region of rabbit

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. The model of the burn wound was produced according to Hoekstra standard (Brans *et al*., 1994) (Fig. 1).

In the treatment group, as a daily procedure, wounds were washed with normal saline. Subsequently, 5 mL of alcoholic extract of garlic 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 non-adhesive 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 1, 7, 14 and 21 days of the experiment.

Statistical analysis: 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 $p<0.05$ (SPSS for Windows, release 10.0.1, SPSS Inc.).

RESULTS

Healing process of experimental burn wounds was without any major complications in both groups during the study. Throughout the days after the experiment the animals showed a normal reaction to the environment and displayed no signs of suffering due to burn wounds.

The clinical evaluation showed visible and significant differences in the process of wound healing after applying the above mentioned medications ($p<0.05$). The rates of wound contraction (percent decrease of wound area) in experiment and control wounds are shown in Table 1.

Results of microbiological examination were showed significant differences between both experimental groups ($p<0.05$) (Table 1). The total number of the bacterial strains on the skin was $4.8\pm0.6\times10^4$ CFU (Colony Forming Unite) cm^{-2} in control group and $3.9\pm0.8\times10^4$ CFU cm^{-2} in the experiment group during the microbiological examination. At this period, the growth of *S. pyogenes*, *S. aureus*, *Escherichia coli* and *Candida albicans* strains were observed.

In the garlic treated group, the numbers of microbes were $7.4\pm0.4\times10^4$ CFU cm^{-2} in the 1st day of the experiment. This amount was $4.2\pm0.2\times10^4$ CFU cm^{-2}, on the 7th day of the experiment. On day 14th day this value was $3.4\pm0.2\times10^4$ CFU cm^{-2}. The total number of isolated bacteria was $2\pm0.6\times10^4$ CFU cm^{-2} on the 21st day. In this group, *Staphylococcus aureus* was isolated on 21st day of the experiment.

In the control group, the total bacterial count of the wound surface in the 1st day, were $8.2\pm0.2\times10^4$ CFU cm^{-2}. On day 7, this value gradually increased during the following days to $9.4\pm0.2\times10^4$ CFU cm^{-2}. This variable was decreased ($7.2\pm0.2\times10^4$ CFU cm^{-2}) at the 14th day. The total count was reached to $5.4\pm0.4\times10^4$ CFU cm^{-2} at 21st day. In this group, on the 21st day, just *S. pyogenes* was isolated.

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

	Day			
Group	0	7	14	21
Experiment	544.2±64.22 (00.00)	280.82±44.02* (48.39)	44.24±25.42* (91.87)	4.22±2.44* (99.22)
Control	562.2±24.04 (00.00)	374.42±12.04 (33.40)	165.22±26.06 (70.61)	66.44±24.14 (88.18)

*Significant difference ($p<0.05$)

DISCUSSION

The goal of this investigation was evaluation of the potential enhancing effect of alcoholic extract of garlic on healing of burn wound from clinical and microbiological aspects. Garlic and its constituents have *in vitro* activity against some important pathogens (Kumar and Berwal, 1998; Ankri and Mirelman, 1999; Harris *et al*., 2001; Yin and Cheng, 2003; Benkeblia, 2004; Yano *et al*., 2006; Sofia *et al*., 2007). Products from garlic have been shown to have antibacterial, antifungal, antiviral and antiprotozoal activities (Harris *et al*., 2001). It was indicated that the antibacterial activity of garlic were due to thiosulfinates, particularly allicin, which is responsible for most of the antimicrobial activity as well as its flavor and aroma (Amagase *et al*., 2001; Harris *et al*., 2001; Yin and Cheng, 2003; Baghalian *et al*., 2005). Allicin was described as a colorless oil, extremely pungent, that characterized the principle odor and taste of garlic. It was reported that allicin in concentrations of 1:85.000 in broth was bactericidal to wide variety of gram-negative and gram-positive organisms. Investigations have shown that extracts from *Allium bulbs* inhibit growth and respiration of pathogenic fungi and bacteria. Alcoholic extracts from fresh garlic bulbs at levels of 3, 5, 10% and inhibited the growth of *Bacillus cereus* on nutrient agar plates by 31.3, 58.2 and 100%, respectively.

Recently, it has been reported that onion and garlic extracts exert bactericidal effects towards *S. mutans* and *Streptococcus sobrinus* and *Porphyromonas gingivalis* and *Prevotella intermedia* (gram-positive bacteria), considered as the main bacteria responsible of dental caries and adult periodontitis, respectively (Bakri and Douglas, 2005).

Epidemiological studies have demonstrated that DAS and DADS from garlic can protect against the *Helicobacter pylori* infection and therefore, to reduce the risk of gastric neoplasia, since *H. pylori* is deeply involved in stomach cancer development (Cellini *et al*., 1996; You *et al*., 1998).

The results of microbiological evaluation of this investigation were showed total bacterial count of wound surfaces in garlic treated group ($2\pm0.6\times10^4$ cfu cm^{-2}) was lesser than surfaces of the control wounds ($5.4\pm0.4\times10^4$ cfu cm^{-2}) at 21st day of this study (Table 1).

Methyl Sulfonyl Methane (MSM) is a naturally occurring sulfur compound found in a variety of foods, including onions and garlic. It is an important nutrient and is essential for the maintenance of connective tissues, joint function, proper enzyme activity and hormone balance (Saifzadeh *et al*., 2006). Sulfur is very important for the formation of collagen and is a major component in

the synthesis of cartilage and connective tissues. It is essential part of keratin which is necessary for the maintenance of healthy situation of the skin, hair and nails. MSM can also decrease scar tissue by changing the cross linking process in collagen to allow tissue repair and healing to take place (Naguib, 2002).

The clinical findings of the present study were showed wound surfaces in the experiment (garlic-treated) group were significantly decreased in comparison to the control wounds ($p<0.05$) (Table 1).

In spite of the topical use of garlic as naturopathic and traditional therapy appears to be widespread (Chowdhury *et al*., 1991; Harris *et al*., 2001; Sivam, 2001; Shukla and Taneja, 2002; Dietz *et al*., 2004; Shadkchan *et al*., 2004), but there are few researches on the accelerating effect of garlic on wound healing (Saifzadeh *et al*., 2006; Sardari *et al*., 2006a, b). These reports have been limited to efficacy of topical application of garlic on incisional wounds while this study investigated effect of garlic extract on burn wound from microbiological view point.

The results of the present study (clinical and microbiological) were demonstrated that the status of wound healing process (the rate of wound contraction and the total bacterial count of burn wound surface) was affected significantly by alcoholic extract of garlic treatment.

With attention to these results, alcoholic extract of garlic can be considered as an available and inexpensive topical agent for accelerating and enhancement burn wound healing. Notwithstanding, there are requirement to illuminate other possible mechanisms involved in the burn wound healing, particularly to some side effect and toxicity due to usage of garlic extract.

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