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Enhancing Effect of Aqueous Garlic Extract on Wound Healing in the Dog: Clinical and Histopathological Studies

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Abstract: November 13, 2006This study was performed to evaluate the enhancing effect of garlic (1% aqueous extract) on the wound healing in an animal model. Five apparently healthy mongrel dogs of both sexes, weighed 20-25 kg were studied. Two rectangular (2× cm^2) wounds were created on the left (experiment) and right (control) caudodorsal areas of each dog. The experiment wounds were treated with 0.5 mL of 1% aqueous garlic extracts every 3 other day for 30 days. The wounds were photographed and compared for rate of wound contraction with digital scanning software. Specimens were taken for histopathological examinations on day 30. Significant differences were seen between the experiment and control wounds for the rate of contraction. On histopathology re-epithelization was more pronounced and the collagen fibers were also more orderly arranged in the experiment group. It is concluded that topical application of aqueous garlic extract, as used in this study could improve rate of wound healing in the dog.

Key words: Garlic, aqueous extract, wound healing, dog

INTRODUCTION

Garlic (*Allium sativum Linn.*), belonging to the Liliaceae family, is a common food spice, used widely in many parts of the world. The center of origin of garlic has been considered to be central Asia. From the center of origin, garlic has been spread west, south and east[1]. In Iran, cultivation and consumption of garlic has a long history and areas under its cultivation are estimated about 10000 ha.

For many centuries various species of genus Allium have been used as vegetables and spices and as folk medicines for the curing of various diseases[2]. The powerful and unusual flavors of many of these plants and their possible medical applications have attracted the attention of plant physiologists and chemists[3-6]. It has been cultivated for centuries all over the world on account of its culinary and medicinal properties[7,8]. Many of the spices and herbs used today were known to the people of the ancient cultures throughout the world and they were valued for their preservative and medicinal powers as well as their flavor and odor qualities[9]. Most of its prophylactic and therapeutic effects are ascribed to specific oil- and water-soluble organosulfur compounds, which are responsible for the typical odor and flavor of garlic[10,11]. Studies were carried out on the antioxidant and antimicrobial properties of garlic and its derivatives such as essential oil and oleoresin[6, 9]. Garlic has also benefits in lowering total plasma cholesterol, reducing blood pressure and decreasing platelet aggregation[1]. In particular, garlic contains the cysteine S-conjugate sulfoxide L-alliin [CH_2 = $CHCH_2S$ (O) CH_2CH (NH_3^+)CO_2], a nonodorous allylsulfinothiolated derivative of cysteine, that is transformed exogenously into several odorous allylpolysulfide analogues when the bulb is crushed, minced, or damaged[12]. These bioactive components have been isolated from aqueous, ethanolic and fermented extracts of crushed garlic and have the potential to interact with a number of cellular targets, particularly those exhibiting reactive sulfhydryl moieties, whose functions range from control of cell cycle to expression of crucial antioxidant and detoxification enzymes. Interactions with these processes may underlie garlic's putative therapeutic potential[12].

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Despite garlic widespread health use throughout history and around the world and the cherished belief that it helps in maintaining good health, warding off illnesses and providing more vigor, however, no work has yet been reported on the effects of garlic on wound healing. Therefore, the objective of this work was to evaluate garlic in form of aqueous extract for promotion of wound healing in the dog as an animal model.

MATERIALS AND METHODS

Preparation of garlic extract: The garlic extracts were prepared fresh everyday. Fresh garlic was purchased from a local vegetable market (Urmia, Iran). Then the garlic bulbs were peeled and ground to form a paste in 1 gram quantity. The paste was then dissolved in 100 mL distilled water in sterile tube. The solution was then centrifuged at 6000 rpm for 20 min at room temperature. The pellet was discarded and supernatant was diluted 10 times with distilled water to get 1% of garlic extract. The concentration of garlic extract used was based on Shukla and Taneja[13].

Animals: Five apparently healthy adult mongrel dogs of both sexes, weighed 20-25 kg were used in this study. The dogs were housed in individual cages and had access to water and food ad libitum. Procedures involving animals and their care were conducted in conformity with the guidelines of the higher committee of veterinary clinical sciences of the institution. Food was withheld for 12 h before surgery. Dogs were premedicated with acetylpromazine (Hoogsrraten, Belgium) (0.1 mg kg^{-1}, intravenously), anesthetized with sodium thiopental (Biochemie GmbH, Vienna, Austria) (10 mg kg^{-1}, 2.5%, intravenously) and maintained with halothane (ICI Pharmaceuticals, Cheshire, England) in oxygen in a semiclosed circle system. Lactated Ringer's solution (Shahid Ghazi Co., Tabriz, Iran) (10 mL kg^{-1}/h, intravenously) was administered during the surgical procedure. Dogs were positioned in ventral recumbency and the caudodorsal regions were clipped and prepared for aseptic surgery.

Experiment schedule: Using a sterile skin marker and millimeter ruler, two rectangular skin defects of 2x3 cm^2 were created at the caudodorsal region of each dog. Left side defect assigned to as experiment and the right one as control, so that each animal served as its own control. The experiment wounds received 0.5 mL of 1% aqueous garlic extract, in aseptic conditions, every three other days for 30 days. Neither prophylactic nor postoperative antibiotic therapy was done. Postoperative analgesia for the dogs was provided by administration of butorphanol (0.4 mg mL^{-1}, IV) at extubation and repeated as needed for 12 h postoperatively.

Assessment: The wounds were photographed on days 0, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30. 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.

Wound specimens of both sides were also taken on day 30 under general anesthesia and fixed in 10% neutral buffered formalin for subsequent histopathological studies.

RESULTS AND DISCUSSION

Wound healing was uneventful during the experiment period. All wounds had similar pattern of healing. No difference in cosmetic appearance was noted between experiment and control wounds. The rates of wound contraction (percent decrease of wound area) in experiment and control wounds are shown in Table 1.

The results in Table 1 showed that the decrease in wound area was significantly greater from day 6 on in the experiment wounds compared to the control ($p<0.05$). Furthermore, although contraction was steadily moved on in both groups, the rate of contraction (percent decrease of wound area) was obviously greater in the experiment wounds.

Microscopically, re-epitheliazation of the wound area was accelerated by the topical treatment with garlic, the epithelial thickness was uniform and normal compared with the control wounds Fig. 1 and 2. The collagen

Table 1: Comparison of wound area (mm^2) (mean±SD) and percent decrease in the experiment (aqueous garlic extract-treated) and control wounds (n = 5 dogs)

Day											
Group	0	3	6	9	12	15	18	21	24	27	30
Experiment	677.9±52.27	574.17±51.46 (15.3%)	340.9±51.21* (49.71%)	178.06±44.26* (73.73%)	86.07±35.48* (87.3%)	50.52±24.47* (92.55%)	38.24±16.92* (94.36%)	19.49±6.82* (97.12%)	7.23±2.67* (98.93%)	3.62±1.21* (99.47%)	2.45±0.92* (99.64%)
Control	655.5±47.85	570.4±63.59 (12.98%)	472.0±34.18 (27.99%)	268.7±35.90 (59%)	161.04±45.32 (75.43%)	109.14±36.42 (83.35%)	65.74±16.47 (89.97%)	39.59±16.82 (93.96%)	12.12±4.21 (98.15%)	9.53±1.42 (98.55%)	7.24±0.98 (98.89%)

*Significant difference ($p<0.05$)

Fig. 1: Photomicrograph of the wound section in control wounds shows normal granulation tissue (H and ×400)

Fig. 2: Photomicrograph of the wound section in experiment wounds shows more advanced maturation of granulation tissue with respect to collagen formation (H and ×400)

accumulation in both sets of wounds was equal, but in the experimental wounds was more orderly arranged. Furthermore, the cellularity and inflammatory reaction of the scar were significantly greater in the control wounds.

Although the topical use of garlic as a naturopathic remedy appears to be widespread[14], evidence-based information on the boosting effect of garlic on wound healing is lacking. Since garlic contains various biochemically active substances and since its extracts have been known to protect organs from various injuries[15], we investigated the potential enhancing effect of aqueous garlic extract on wound healing which is not studied so far.

The results of the present study demonstrated that the rate of wound healing and the wound microstructure were affected significantly by 1% aqueous garlic extract treatment.

Several methods of garlic application persist in the folk remedies of many cultures as treatment for a variety of illnesses. These include tincture of garlic in oil used for treatment of ear inflammation as well as topical garlic application for treatment of asthma, skin lesions, or as an antimicrobial agent[14].

Most of the medicinal effects of garlic are referable to its sulfur-containing compounds[1]. By use of gas chromatography, mass spectrometry and high performance liquid chromatography, allicin, diallyldisulfide, 1, 3-dithiin and other related disulfides were identified in water extract of garlic[16]. Methyl Sulfonyl Methane (MSM), also known as dimethyl sulfone, is a naturally occurring sulfur compound found in a variety of foods, including onions and garlic. It is also found in human body fluids, tissues and urine. MSM, which has the chemical formula $C_2H_6O_2S$, is an important nutrient and essential for healthy connective tissues and joint function, proper enzyme activity and hormone balance. Sulfur is vital for the synthesis of collagen and is a major component in the formation of cartilage and connective tissues. Sulfur is present in keratin, which is necessary for the maintenance of the skin, hair and nails; and it gives strength, shape and hardness to their protein tissues. MSM can also reduce scar tissue by altering the cross linking process in collagen to allow tissue repair and healing to take place[17]. We assumed that the enhancing effect of aqueous garlic extract on wound healing may be attributable to its MSM content.

In view of the fact that the present experiment is the first study on the garlic as a promoter of wound healing, our results are not comparable w ith those of previous works.

Under the light of these explanations, local application of garlic causes significant improvement in the rate of wound contraction, which is in conformity with the microscopic evaluation of the wound specimens in this study.

CONCLUSION

To sum up, our results suggest that aqueous garlic extract may be used to accelerate the process of wound healing in the dog. However, it seems further studies are required to clarify other possible mechanisms leading to this and to evaluate the effects of various doses of garlic extract during different periods of time.

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