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Study the Effect of Aqueous Extract of Garlic (*Allium sativum*) on Healing Procedure of Burn Wound on Rat

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HIGH antimicrobial and antioxidant effects of garlic make it possible to use as an effective topical cream to heal burn wound injuries. The present research was done to evaluate the burn wound healing effects of the aqueous extract of garlic on rat model. Aqueous extract was achieved from fresh garlic. Thirty healthy adult rats were subjected to burn wounding and then randomly treated with silver sulfadiazine (positive control), cream base (negative control) and garlic topical cream (0.8%, 0.4% and 0.2%). Rats were then maintained for 21 days and macroscopic and microscopic examinations were performed through the experiment. Burn wound contraction was increased in all groups of rats during the experiment period. The highest percent of burn wound contraction at day 21 was found in rats treated with 0.4% garlic topical cream (88.10%), while those treated with cream base had the lowest wound contraction (70.30%). Rats treated with 0.4% garlic topical cream showed more rapid granulation, formation of scar and healingin the macroscopic examination. Additionally, rats treated with 0.4% garlic topical cream had the higher amounts of tissue granulation with dense collagen deposition in the microscopic examination after 21 days of wounding. Burn wound healing effects of the garlic extract was not dose depended. It seems that application of 4% garlic cream is an effective way to improve the burn wound healing of rats. However, supplementary studies are needed to evaluate other effects of garlic cream on burn wound.

Keywords: Garlic, Aqueous extract, Burn wound, Healing, Rat.

Introduction

Burn wound healing is one of the most serious matters facing medical practitioners. Burn wounds are common superficial and deep injuries amongst both humans and animals all around the world. Nevertheless, burn constitutes a main health issue in developing countries due to the considerable incidence of difficulties and also restriction of therapeutic and financial resources [1]. The mean annually incidence rate of burn is around 1.30 million cases in advanced countries like the United States [2]. Additionally, around three million cases are raised to hospitals because of the occurrence of burn yearly [3].Wound healing, re-epithelization and also prevent from occurrence of infections are the most significant aims of burn management [4].

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Using topical ointments and creams locally in the site of burn wound injuries is the best medical and clinical way to prevent from occurrence of infections, deformities and mis-formation. Silver sulfadiazine is the topical cream of choice in unadorned cases of burns and is used nearly globally today in partiality to components including mafenide acetate and silver nitrate. The silver sulfadiazine ointment, although being operative, origins some general complications such aserythema multiforme, neutropenia, methemoglobinemia and crystalluria[4]. It is also ineffective in severe burn injuries. Thus, its extensive application has been prohibit especially in the cases of severe burn injuries [4].

Near to 80% of the human population of developing countries and half of the population of developed countries used traditional medicine in their health care[5, 6]. Garlic (*Allium sativum*) is one of the most widely used medicinal plants with high therapeutic effects. Garlic belongs to the Liliaceae family, is a common food spice, enthusiastically used in many sites of the world including Asian, African, European and some parts of the American continents [7, 8].Otherwise, garlic is a broadly distributed plant used in all parts of the world not only as a spice but also as a prevalent medicinal plant remedy for numerous diseases and disorders including wounds, infections, ulcer, diabetes and blood pressure for thousands of years[7, 8]. Garlic has also severe antimicrobial, antioxidant, anti-inflammatory, anticancer and aging prevention effects [7, 8]. Edible nature and antimicrobial and antioxidant activities of garlic make it appropriate burn and wound healing candidate [7-10]. Numerous recognized investigations described the high wound healing effects of different species of garlic [9, 10].

Considering the high incidence rate of burn injuries in Iran and also lack of coded research on the role of Garlic extract in burn wound healing, the contemporary investigation was carried out to study the burn wound healing effects of the aqueous extract of garlic on rat model.

Materials and Methods

Ethics

The current survey was performed according to Laboratory Animal Ethics Charter of the Alborz University of Medical Science to decrease laboratory animal pains.

Animals

Thirty healthy adult Wistar male rats (250–280 g) were housed one per cage and maintained under standard housing conditions (room temperature 24-27 °C and humidity 6065%- with 12:12 light: dark cycles) for a week prior to the experiment to acclimatize. Additionally, the anie mals had free access to feed and water. All rats were purchased from the Pasteur Institute (Tehran, Iran). All procedures were conducted using facilities and protocols approved by Institutional Animal Ethics Committee of Alborz University of Medical Sciences.

Burn wound model

Rats were anesthetized using an intraperitoneal injection of ketamine (50 mg/kg, Alfasan International, Woerden, Netherlands) and xylazine (10 mg/kg, Alfasan International, Woerden, Netherlands). Their back hairs were shaved by electrical clipper and the skins was washed with alcohol 70% (ethanol, Merck, Germany) and rinsed with sterile water. Second degree burn wound was created using the 2 cm diameter brass plate was heated on flame of alcohol for 40 s and then pressed onto the skin of rats with a certain force for 12 s.

Plant materials

Fresh garlic (*A. sativum*) was prepared from Babol, Iran during autumn 2018. Fresh garlic was identified and approved by Herbarium Department of Pharmacognosy of Shahid Beheshti University of Medical Science (HSBU, Tehran, Iran). The voucher specimen (No. 270) is preserved in the herbarium of this department for reference. The raw garlic cloves were peeled, chopped into small pieces and blended. The aqueous extract was prepared by adding 250 ml of distilled water to 50 g of garlic powder and the resulting solution was incubated for 24 h in 25 °C in a shaker (250 rpm). The extract was then filtered with Whatman filter paper (No. 2).Then, the filtrate solution was completely dehydrated for 8 to 10 hours in water bath to provide a crude extract with 20% yield [11].

Preparation of garlic cream

After extraction, liquid extract was sterilized using the UV lamp for about 24 h. Sterilized garlic extract was subjected to freeze dryer device. Aqueous garlic extract was prepared at 0.2, 0.4 and 0.8 percent concentrations using dissolving of garlic extract in sterile distilled water and then mixed with sterile cream base (cold cream base) (Farabi base cream, Tehran, Iran). Cold cream was only used as an ointment base and carrier of garlic (Fig. 1) [12].

Fig. 1. A samples of garlic cream produced from the mixture of garlic aqueous extract and cold cream.

Treatments

Rats were randomly classified into 5 different groups of positive control (treated with silver sulfadiazine topical cream (Flamexin®, 1%, Sinadaru, Tehran, Iran)), negative control (treated with cold cream (cream base)), G1 (treated with garlic topical cream (0.8%)), G2 (treated with garlic topical cream (0.4%)) and G3 (treated with garlic topical cream (0.2%). All groups contained 6 rats. All arts were remained up to the end of the study. Cold cream was used to prepare G1, G2 and G3 creams. As this procedure was done in a daily manner, there were no need for any therapeutic stability tests.

Burn wound analysis

All wounds were washed with normal saline in on daily basis, and wounds in all groups received their appropriate cream and were dressed. Macroscopic examination of burn wound in different groups of rats was studied using the photographic camera (Canon, G7, Japan). All wounds were treated with each therapeutic options one time in each day. Procedure was done rendering method labeled beforehand [13, 14]. Macroscopic images were taken in days of 3, 9, 12, 18 and 21 after burn wound[13]. Wound areas were measured manually and calculated in square millimeters [13]. We measured the lengths of the major axis and the minor axis of the wound on days 0, 3, 5, 8, 10, 12, and 15 after wounding [14] with a verniercalliper[15] according to principles introduced beforehand.

The measurement of the wound area was calculated by the formula for an ellipse([0.5 × the length of the major axis][0.5×the length of the minor axis] (16] (π]. The wound healing rate was calculated as follows: (Area of original wound-Area of remaining wound)/Area of original wound×100 [17].

Histopathologic examination

All histological procedures were performed by an independent blind observer. From each sample in the last day, two slides were prepared using sterile scalpel and tissue forceps. Burned skin tissue samples were taken for histological studies with a small excision containing part of the wound area. Tissue samples were fixed in 10% formalin solution (Merck, Germany). Paraffin embedded tissue section of 4 µm were prepared and stained with hematoxylin and eosin (H & E). Light microscopy was used to assess pathological changes [18].

Each slide was given a histological score ranging from 1 to 12, with 1 associated to no healing and 12 associated to a completely re-epithelialized wound, according to the method described by Greenhalgh et al. [19].

Statistical analysis

Data were transferred to the Excel software and described as mean and standard deviation (Mean ± SD). Statistical comparisons between groups were carried out using SPSS (version 19.0; SPSS Inc., Chicago, USA). One-way ANOVA test

was used to analyze data. A P value ≤ 0.05 was considered as statistically significant.

Results

A total of 30 burned rats were subjected to treat with different concentrations of garlic topical cream (0.8, 0.4 and 0.2%). Table 1 represents the percent of burn wound contraction in rats treated with different concentrations of garlic cream and also the control group during the experiment period. There were no obviously burn wound contraction at the first day of the experiment (0%). Percent of burn wound contraction was increased in all groups of rats during the experiment period. The highest percent of burn wound contraction at day 3, 6, 9, 12, 15 ,18 and 21 were found in rats treated with 0.2% garlic cream (G3) (31.90%), G3 (37.40%), G3 (33.40%), 0.4% garlic cream (G2) (45.90%), G2 (57.30%) , G2 (71.40%) and G2 (88.10%), respectively. The highest and lowest percent of burn wound contraction at day 21 were found in rats of G2 group (88.10%) and those treated with cream base (negative control) (70.30%), respectively.

Figure 2 represents the macroscopic features of burn wounds in each studied groups. The processes of granulation, formation of scar and healing are also shown in this figure. The procedures

TABLE 1. Percent of burn wound contraction in rats treated with different concentrations of garlic cream and also the control group during the experiment period.

Day of experiment	Burn wound contraction in different groups (%)				
	G1	G2	G3	Positive control	Negative control
1	0	0	0	0	0
3	26.70	19.20	31.90	16.60	24.10
6	33.56	28.20	37.40	30.10	33.90
9	29.90	29.50	33.40	28.10	22.60
12	29.70	45.90	27.90	24.00	24.60
15	47.40	57.30	36.70	25.00	33.00
18	67.00	71.40	61.80	51.60	52.90
21	79.30	88.10	83.40	71.50	70.30

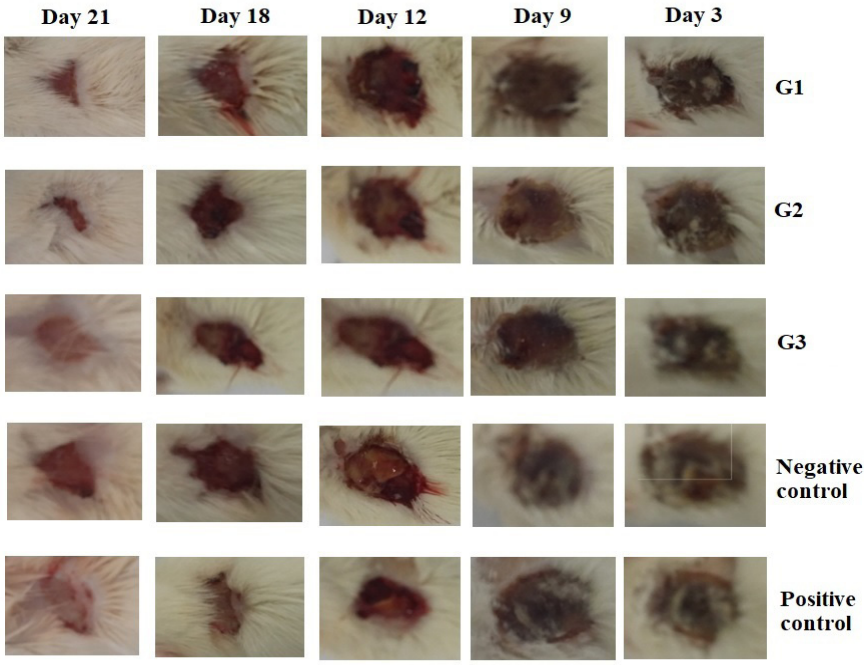

Fig 2. Results of the macroscopic examination of burn wound healing in rat model.

of granulation, scar formation and healing were more rapid in rats of the G2 group. Additionally, the area of burn wound was lower in rats of the G2 group.

Figure 3 represents the results of the microscopic examination of burn wound healing in rat model. Micrographs of burn wounds were studied using the H&E at 21 days after wounding. At 21 days after wounding, rats of the G2 group had the higher amounts of tissue granulation with dense collagen deposition (Fig.3). The control group showed focal ulceration and moderate inflammation and re-epithelialization (Fig.3).

Figure 4 represents the occurrence of granulation tissue and collagen in rats of the G2 group.

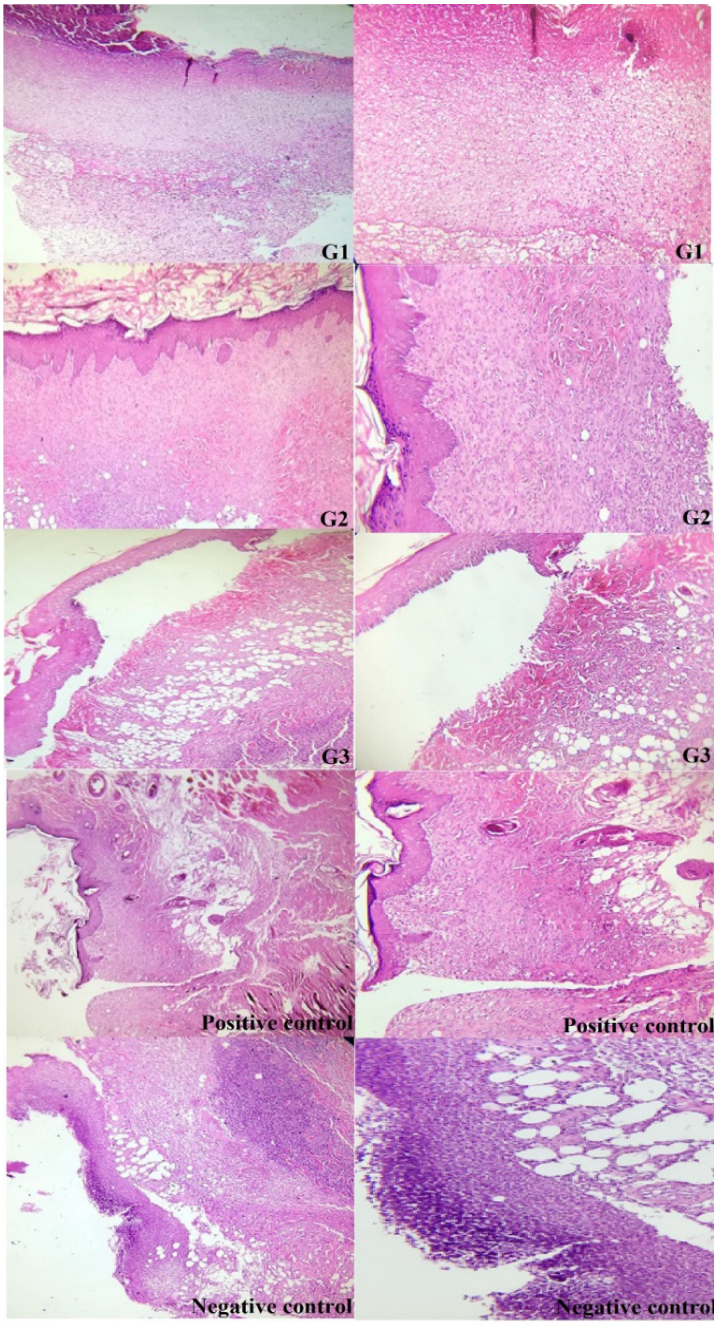

Fig.3. Microscopic examination of burn wound healing in rat model at 21th day after wounding using H&E staining.

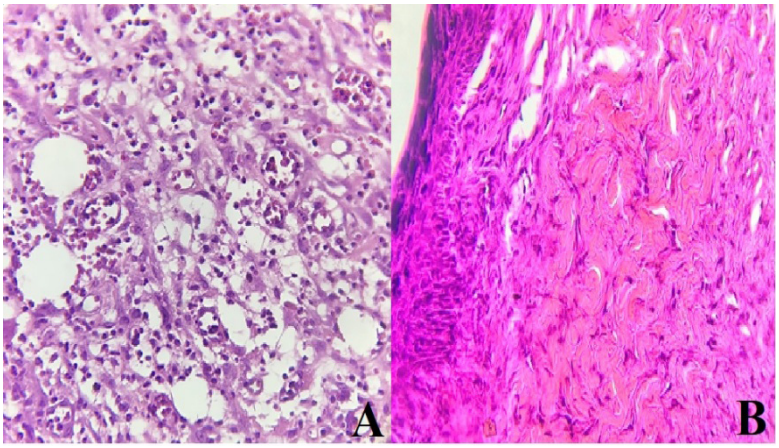

Fig. 4. Granulation tissue (A) and collagen (B) in rats of the G2 group.

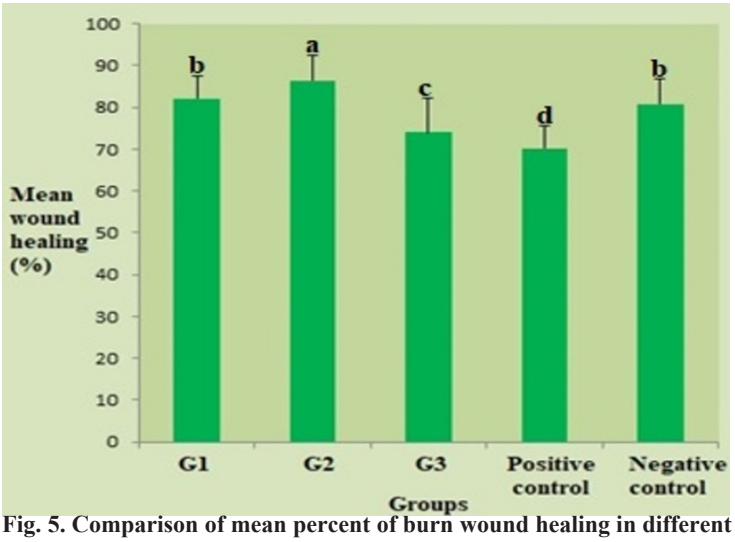

Fig. 5. Comparison of mean percent of burn wound healing in different groups of rat on day 21. Dissimilar letters represent statistically significant difference about *P*<0.05.

Figure 5 represents the comparison of mean percent of burn wound healing in different groups of rat on day 21. Rats of the G2 group had the highest percent of the burn wound healing, followed by G1 and negative control.

Discussion

Burn is the most widespread and imperative type of trauma. Its therapeutic choices can accelerate healing of injured skin and avoid from the occurrence of infection and deformities. Researchers have established that infections in the locations of injured skin are main reasons of mortality in hospitalized patients with extensive burns. Then, using local ointment with considerable antimicrobial effects can diminish the risk of burn wound infections and abridge the period of treatment [19].

The current research was done to evaluate the burn wound healing effects of the aqueous extract of garlic on rat model. Results obtained from this research showed that application of 0.4% garlic cream had the highest wound contraction (88.10%) at the end of experiment period. Furthermore, macroscopic examination revealed that the area of the wound in the group treated with 0.4% garlic cream (G2) was entirely lower than other tested rats in all days of experiment. Moreover, microscopic examinations of wounds revealed that rats treated with 0.4% garlic cream (G2 group) had new epithelial tissues in earlier stages of healing and their wounds were covered with new epidermis. In some

areas generation of the sebaceous and sweat gland with complete tissue re-epithelialization, fibroblastic proliferation and finally modeled dense collagen mesh, and moderate fibrosis were also observed in rats treated with 0.4% garlic cream (G2 group).

Garlic has been exposed in numerous researches to progress wound healing, and it has been resolute that allicin, the molecule accountable for garlic's spicy scent, is the active compound. Allicin exhibits its antibacterial activity through sulfhydryl modification of bacterial proteins especially in *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria which are responsible for burn wound infections [20]. Additionally, the allicin is a key factor in fibroblast activation; this leads to more rapid approximation of skin edges, more organized collagen deposition, and more rapid upsurge in tensile strength of the healing tissue and subsequent wound healing [21]. Ejaz et al. [21] testified wounded chicken dorsum skin treated with aged garlic solution exhibited an increase in the number of new loosely packed collagen and maturation of collagen bundles at day 4 and 6 post wounding, respectively. Saifzadeh et al. [22] accompanied a comparable research on dogs and conveyed that the collagen fibers were more orderly arranged in the wounds treated with aqueous extract of garlic. They also revealed that aqueous extract of garlic caused decrease in the epithelial gap and collagen synthesis. A study which was conducted by Jalali et al. [23] described that application of garlic extract had a significant effect on the rate of wound contraction in rabbit model.

Several investigations revealed that the aqueous extract of garlic had considerable antioxidant effects which increase its burn wound healing activities [24, 25]. Presence of antioxidant agents caused significant decrease in free radical production and also prevent from the occurrence of deformities. Aqueous extract of garlic has antioxidant, anti-hypertrophic and anti-apoptotic effects and thus can easily prevent from the occurrence of deformities and mis-formation in newly produced epithelial cells of injured tissue [26]. Antioxidant agents are essential for synthesis of collagens, inhibiting inflammation and angiogenesis. It was recorded that the garlic-derived chemo-preventive agent allicin is strong radical-trapping antioxidants in lipid bilayers. It was specified that there may be a role for garlic extract in resolving inflammation by persuading the apoptosis of responding immune cells, as the need for them is reduced [27]. There was an evidence for effect of garlic extract by shortening the inflammatory phase and maturation of collagen bundles in treated burn wounds [28].

Similar investigations have been conducted in this field. Fares et al. [29] conveyed the obvious regeneration of the epidermis and dermis with noteworthy upsurge in hair follicles number and multiple immunostained CD44 +ve cells in rats treated with garlic extract. Farahpour et al. [30] stated the substantial improvement in fibroblast, fibrocyte, and mast-cell production in rats treated with 2% garlic extract. Additionally, higher neovascularization was found on day 3 after wounding in rats treated with 2% garlic extract. They also concluded that garlic extract indorses wound healing due to its initial influence on mast-cell distribution, which boosted synthesis of collagen and angiogenesis upregulation, and condensed the healing procedure by elevation of the intra-cytoplasmic carbohydrate ratio. Similar reports about the considerable effects of garlic extract on burn wound healing were also described by Vibha et al. [31] (India), Zakiah et al. [32] (Indonesia) and Venâncio et al. [33] (Brazil).

Phytochemical investigations revealed that methyl sulfonyl methane (MSM), also known as dimethyl sulfone, is one of the main chemical components in the garlic extract [34]. Sulfur is vital for synthesis of collagen and is a main component for formation of connective tissues and cartilages. It is also present in keratin which is essential for maintenance of skin. MSM can also decrease scar tissue by altering the cross linking process in collagen to allow tissue repair and healing to take place [34]. Thus, it is not surprising that burned rats treated with aqueous garlic extract show complete burn wound healing at the end of experiment period.

Conclusions

To put it in a nutshell, we identified considerable burn wound healing effects of the aqueous garlic extract on rat model. Findings displayed that 0.4% aqueous garlic extract could accelerate the healing of burn wound in rats. Comprehensive healing of burn wounds on day 21 with respect to the low levels of inflammatory

reactions, neutrophils and bleeding and high presence of differentiated tissues and in some cases hair follicles in the skin layers were confirmed the regenerative effects of 0.4% aqueous garlic extract. Further studies are recommended for evaluation of the other effects of garlic cream on burn wound and also the possibility of its application in the medicine.

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