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Influence of Alcoholic Garlic Extract on Wound Healing of Male New Zealand White Rabbits Full Thickness cutaneous Injuries

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Abstract

Garlic can be used to effectively heal wounds due to its high levels of antioxidants and antimicrobials. This study examined the effects of garlic extract on rabbit models of wound healing. using fresh garlic, extract was produced. The experiment involved sixteen adult male New Zealand white rabbits, weighing between 1±0 and 300 kg, and ranging in age from 9 to 12 weeks. The animals were split up into two major groups at random, with eight animals in each group. Group (A) the skin wounds were excluded from treatment as the control group. In Group (B), the wound was treated by application of 15% garlic extract for only 2 times per day at first 7 consecutive days immediately post wounding . Macroscopic results have shown significant decrease of the wound size ($P \leq 0.05$) after 7 and 14 days, in comparison with the control group in the treated groups .The epithelization period in treated groups were seen at (7) days which was shorter than that of control group (10) days, and the control group have reveald less contraction($P \leq 0.05$) than that of other treatment groups. microscopic examinations results of group A (control) at 7th days PW. appeared infection, congestion, delayed re-epithelization of the wound, severe inflammatory cell infiltration , while at 14th days PW. ,there were abscessation with thick crust, cracked and splintered scab , severe hemorrhage beneath the epidermis, thick and coarse collagen fibers, a little quantity of partial stratum basal hyperplasia and granulation tissue. At With 14 days PW.There were thin crust above the wound which appeard partially detached from epidermis, profuse granulation tissue , complete and thin epidermal layer, marked stratum basal hyperplasia , meeting of wound edges with absence of hemorrhage beneath the epidersmis , formation of new and small haire follicles, the epidermis and thin, regular layer of collagen are covered in neither a crust nor a fully keratinized layer fibers start to develop.

Keywords: Garlic, extract, wound, Healing, Rabbit

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Introduction

At 15% of the adult body weight, the skin is the biggest organ in the body . The epidermis, dermis, and subcutaneous tissue are the three layers that make up the skin. (Kanitakis, J. (2002). serve as a barrier against various infections and physical harm in addition to serving as a sensory organ. Furthermore, it serves as a shield for the body against ultraviolet light and is essential for maintaining life via the control of thermoregulation, hydration and electrolyte balance, and immune system function (William, *et al* (2005). Wounds need to be triggered and recruited by a number of cell types in order to heal. B. fibroblasts, endothelial cells, keratinocytes, and inflammatory cells—macrophages in particular, which appear to be essential to the process—are among them. (Rodero, *et al* (2010) Following the cleansing of the wound site, the fibroblast migration initiates the proliferative phase and deposits fresh extracellular matrix (ECM), the components of which are essential for the antigenic process and proper blood vessel growth. During wound healing, fibroblasts—the primary cell type involved in the regulation of extracellular matrix—are recruited to form a collagen scaffold through a direct mechanical action and the secretion of particular proteoglycans. Fibroblasts also serve as an energy reservoir. Epidermal growth factor (EGF), which is involved in wound healing, modulates their activity. During wound healing, the fibroblast's production of bundles of collagen fibrils is essential for the growth and migration of new blood vessels in the injured regions (Tettamanti, G. *et al* (2004). During the remodeling phase, new collagen is formed. The tensile strength of the wound is determined by the amount of collagen secreted (Blee *et al* (2002). Garlic extract comprises Allicin has a strong antibacterial impact, according to research (Ankri S *et al*.(1999), Barak *et al*. (2007). Angiogenesis is essential for wound healing, and diabetes and venous or arterial insufficiency are associated with a higher risk of chronic wounds due to decreased angiogenesis. Examining the impact of various natural treatments on wound healing is crucial to intervene and enhance wound closure. To find out how different old garlic solution (AGS) concentrations affect wound healing, do a wound test. ascertain how AGS affects angiogenesis, re-epithelialization, dermal matrix regeneration, and wound closure

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(Srimuzipo P *et al(*2009), Jalali FSS *et al*(2009).

Materials and Methods

Animals

Sixteen white newzealand rabbits were divided randomly into two main groups and each group contains 8 rabbits. In Group (A) the skin wounds were without treatment as control group .In Group(B) was treatment by placing and covering of garlic extract with 15%concentration (EJAZ, S. *et al*(2009). for twice day topical application per day and for first 7 consecutive days immediately post wounding. Ethics the current survey was performed according to Laboratory Animal Ethics the University of Al-Qadisiyah College of Medical Science to decrease laboratory animal pains.

Wounding and preparation of wound tissue

After general anesthesia, the incision site (back of the animal) was prepared for aseptic surgery. These wounded rabbits were distributed. randomly. divided into two groups based on the way they were two groups treated. All these animals were induced removal 1 cm a full thickness circular skin incision on the back of rabbits in all groups.. (clipping, shaving and washing the area by hapetin application as antiseptic by gauze saturated with 70% alcohol and application povidine iodine) lateral surface area .A skin incision by across the entire skin thickness (1 cm diameter) was induced using a punching machine and left without treatment for the control group. The incision was treated group B was treatment with garlic extract only.

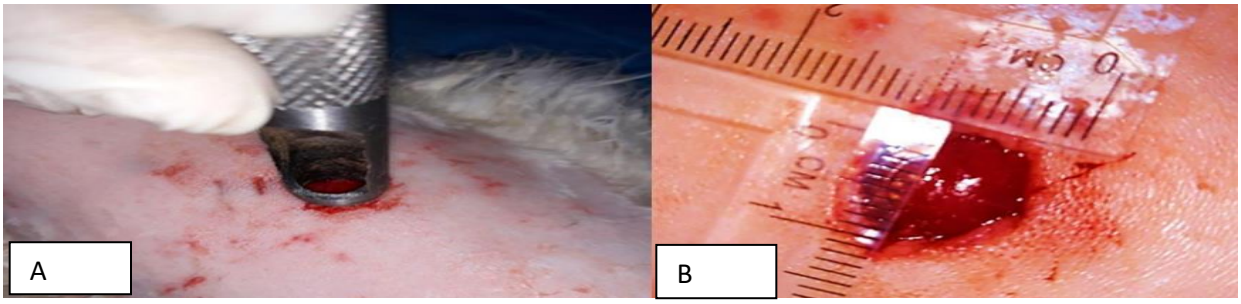

Fig1: photograph shows induction of open wound. Reveal the measuring of this wound in all groups.

Preparation of 0.15 % garlic extract

In March, carnations of Allium sativum were bought at the local market in Iraq. After separating and manually slicing the cloves, around 500 g of fresh onions were kept at room temperature (23–24 °C). After 24 hours of oven drying at 40 °C, 12 g of dry extract was produced from the resultant extract. Prior to usage, the extracts were kept between -20 and −20 °C, as per (Abad, *et al* (2011). Fresh Iraqi garlic was peeled and cut into small pieces after the outer . shell was

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removed. Next, 50 grams of crushed garlic was added to a Soxhlet beaker. Garlic 50g were crushed, placed in beaker contain ethanol 70%of soxhelt apparatus. Dissolve garlic extract 15g in distilled water 100ml. Sterilization of the garlic extract by microfilteration 0.22µm.

Chemical of Garlic extract Analysis

Fourier –transform infrared spectroscopy (FT-IR) is an absorption technique used to identify chemical bonds and molecular structure of the Compounds.in University of Babylon (Moros, J *et al* (2009). The analytical technique known as gas chromatography-mass spectrometry (GC-MS) combines the capabilities of mass spectrometry and gas chromatography to detect various compounds present in a test sample (Sparkman, *et al* (2011) at the University of Basrah.

Macroscopic evaluation of wounds

Using a graduated millimeter measurement ruler, the diameter of the circular wound was first measured on the seventh and fourteenth days after the wound was first created. When the wound's shape changed and it was no longer circular on the fourteenth day, the outer dimensions of the wound—length along the longest axis, or L—and width along the smaller axis, or W—were measured. To avoid contamination, the same instrument was always used, and stringent asepsis measures were implemented (Jorgensen, *et al* (2015), Nichols E. (2015). animal was 1 cm. The size of the wound was measured using two graduated millimeter measuring rulers. the circular surface area (sequar) equals (half diameter) ^{2}x 3.14 on the seventh day (PW) of the experiment.Length x Width is the surface area when the wound's form changes. The metric provided by (Sardari K *et al* (2006). was used to compute the percentage of epithelization/wound contraction/total/wound area. Using the following formula, wound contraction after 7 and 14 days post wounding (PW) was computed as a percentage of the initial wound area size reduction: The percentage of wound contraction is equal to (total wound size on (day/n) - wound area on day n / wound area on day 0 x 100) as a percentage of the original wound. The percentage of epithelization was calculated as follows: %epithelization(day n) = $Aaea\ ob\ epithe\ lizab\boldsymbol{U}n(day\ n) \times 100\ tabn\ wout\ (day)$.(Nagar, *et al* (2016), Belachew, *et al* (2020).

Histological examination of wound healingPreparation of specimens for histological examination Preparation of specimens for histopathological examination.

The anesthetized of animals by general anaesthesia. 1cm of skin full thickness were taken at 7/14 days post wound from all groups, the histopathological evolution was performed on (0,3,7,14)days P.O stains .

A full- thickness incisional biopsy specimens were obtained according to (Falanga V,Saap LJ,Onoff A. (2006) Following preservation in a 10% buffer formalin solution, the specimens were sent to a histopathology laboratory where they were stained with hematoxylin and eosin to assess the collagen deposition, size and developed of skin layers,

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and formation of new blood vessels. (Estevao, *et al* (2019).

Eight animals from each group were slaughtered at 7, 14, and 14 post-operation, and skin samples were obtained for histological analysis in order to assess the degree of skin healing. The parameters of the histologic assessment of wound healing were evaluated during the examination of histopathological slides stained with H&E stain. These included the presence or absence of edema, vascular proliferation, vascular congestion, neutrophilic infiltrate (mild, moderate, and severe), macrophage (mild, moderate, and severe), lymphocytic infiltrate (mild, moderate, and severe), young fibrosis, granulation tissue, and fibroblast proliferation. (Gal, P. *et al* (2009) .

Statistical Analysis:

The ANOVA test and the Least Significant Difference (LSD) were used to statistically evaluate the morphometric data in order to determine the significance between groups at the p≤0.05 level (Sas.statisistical analysis system ,Users guid.statistical.(2012.)

Results

Closure of wounds

Acute Wound Analysis all created wounds of treatment and control group diminished rapidly in size along the examination, yet the close inspection of wounds images indicated that the rate of wound closure in treated wounds weresignificantly (P≤0.05) more along the period of the study as compared to untreated wound. Depending on the information in (Tab.1below),the mean ± SD of total wound area for treatment group in day7 was recorded (27.74± 3.65) cm; and Control group (13.19± 3.21) cm. There were some significant differences between groups. However, total wound area in day 14 in treatment group was significantly higher than in Control group (P<0.05) (43.16± 5.55), (29.84± 3.20) respectively. The mean ± SD of epithelialized area of each group in day 7 was as follows; in treatment and control group recorded (65.16± 3.56) (32.84± 3.20) cm respectively. There was significant differences were determined between treatment and control group (P≤0.05) and showed better wound contraction compared with group Control as follows; in treatment and control group recorded (55.31± 3.72) (35.28± 3.27) cm .

Tab. 1: Shows the Rates of Total Wound Healing (WH %), (cm) in both groups

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GroupDay	Mean ± SE	
	Control Group	Treatment Group
0 th	0.00 ± 00 a	0.00 ± 00 a
3 th	0.00 ± 00 a	0.00 ± 00 a
7 th	BC 13.19± 3.21 b	B 27.74± 3.65 a
14 th	AB 29.84± 3.20 b	A 43.16± 5.55 a

Surface area

On day zero, the initial wound surface area in both the treated and control animals measured 60.30 mm^2. At seven days, there was a significant P ≤0.05 decrease in the surface area (size of wound) between the treated groups and the control group. while the scores of all treatment groups become near each other at 14 days ,but all these treatment groups showed significant difference (P≤0.05) from control group at this time .

Epithelization

Groups A and B experienced an epithelization time ranging from 8 to 10 days, but group B experienced a shorter duration of 8 days compared to the control group's 8 days.

Wound contraction

In comparison to the control A group, the percentage of wound contraction was substantially higher in the treated groups at days 7 and 14 (P≥0.05). Group B recorded the higher significant decrease of wound size (00.00 mm2), and the highest significant percentage of wound contraction (55.31) at 7 and 14 days PW) Table (2) .Fig (2).

Tab2: Shows the Rates of Wound Contraction (WC %),(cm) in both groups

GroupDay	Mean ± SE		LSD
	control Group	Treatment Group	Value
0 th	0.00 ± 00a	0.0 ± 00a	0.00 NS
3 th	0.00± 00a	0.0 ± 00a	0.00 NS
7 th	BC 16.00± 3.21 b	B 27.00± 3.11 a	7.209 *
14 th	AB 35.28± 3.27 b	A 55.31± 3.72 a	8.822 *

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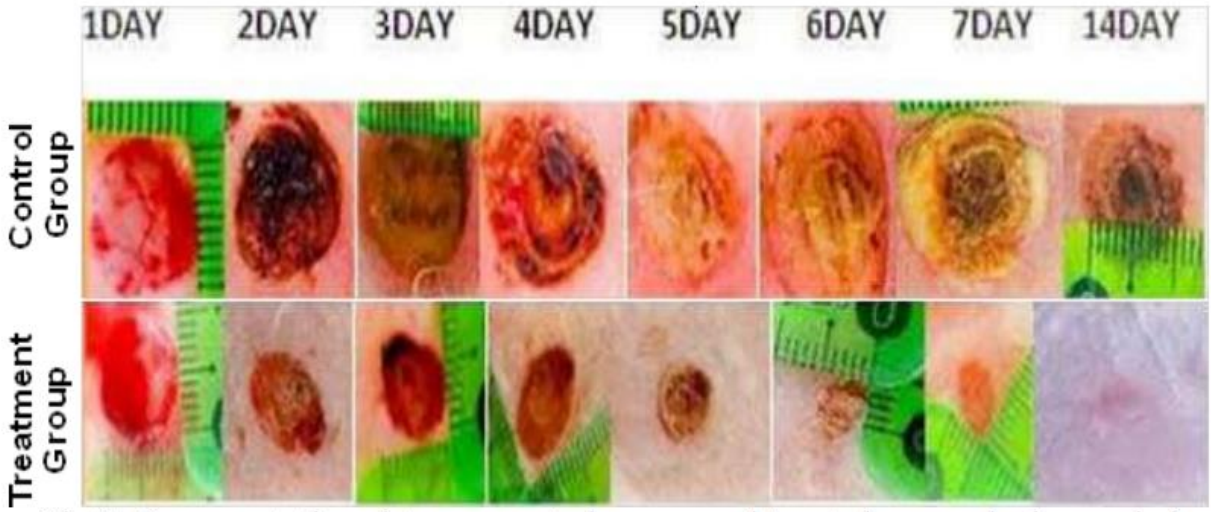

Fig 2: Representative pictures control group and treated group during period experimental study

Histopathological Assessment Of The Wound Healing

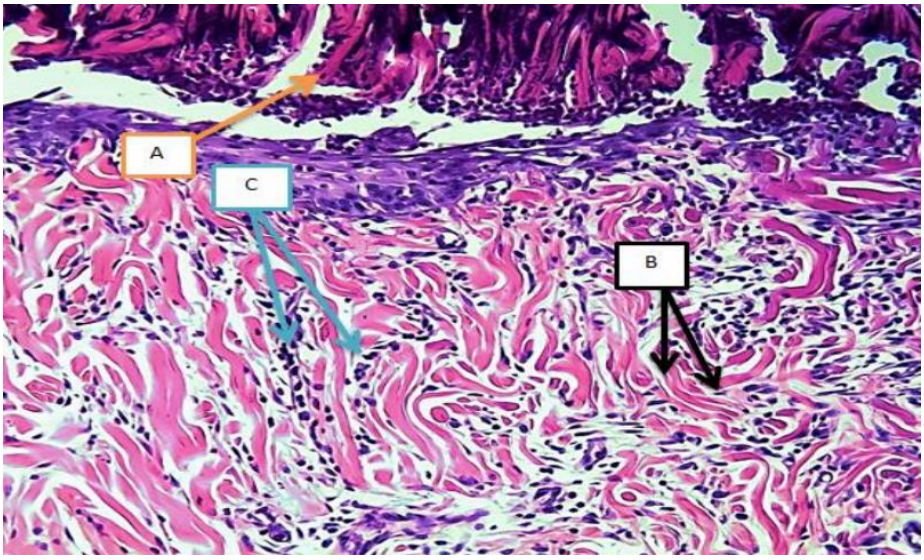

Fig 3: The circular wound in skin of group A displayed a thick crust over the wound region (B) and abcessation (A) at 7 days post-wound. Collagen fibers (C) that are irregular, acidophilic, and interlaced, with a significant infiltration of inflammatory cells in between the fibers.100X H&E

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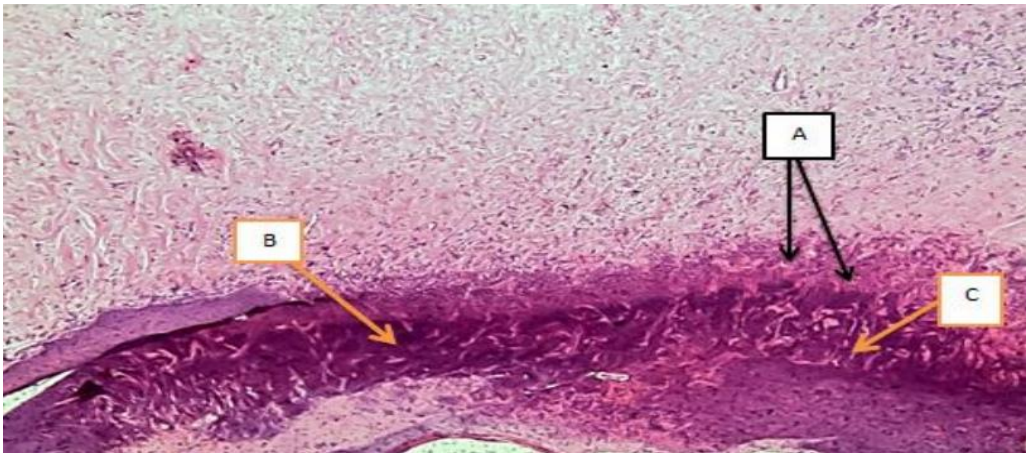

Fig4: Histopathological appearance of circular wound in skin at 14day PW.in group A .(A)severe inflammatory reactions which characterized by (B)high infiltration of inflammatory cells .Also there is thick crust with)C) abscessation in it and this crust appeared connected closely with the skin layers. Healing appears to be very weak with scar tissue. H&E.100X

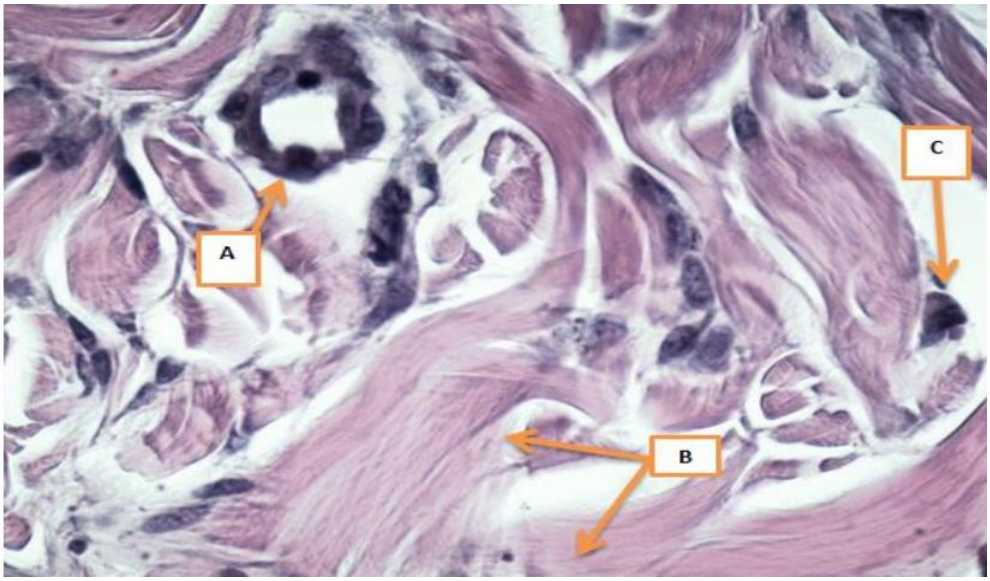

Fig5: Histopathological appearance of circular wound in skin at 7 day PW.in group B showed (A) new formation of new b.v due to proliferation of endothelial cells. (B)Also there was thick and

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interlaced collagen fibers(C) with infiltration of macrophages(C). Higher magnification ;400X H&E

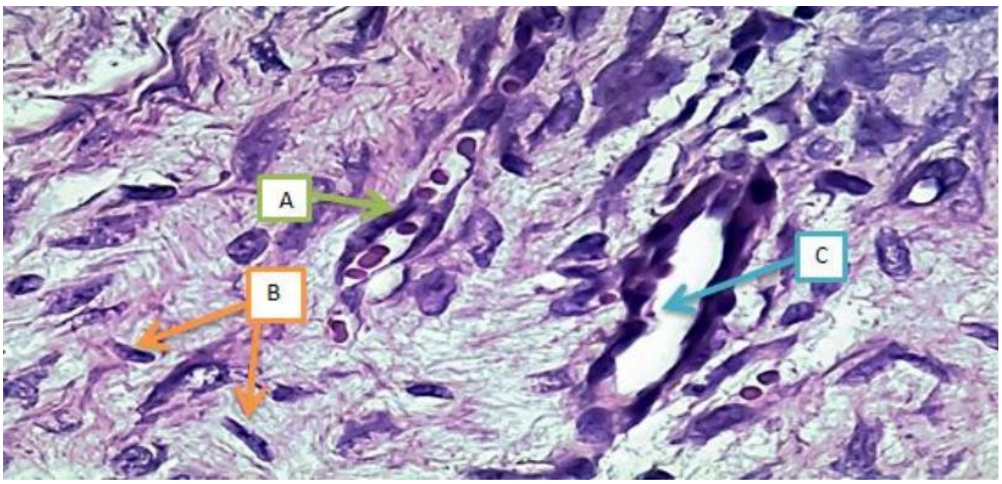

Fig 6: Histopathological appearance of circular wound in skin at 14day PW.in group B showed (A)marked angiogenesis (large and extended newly formed blood vessels(B) which contain red blood cells (C).proliferation of fibroblast horizontally on the wound. . 400X H&E.

Discussion

This study set out to assess the impact of garlic extract on the wound-healing processes of rabbit skin. This study evaluated effects on proliferation, migration, and angiogenesis in vitro and established the resorption capability in vivo in a full-thickness wound rabbit model based on the macroscopic and histological data that we were able to get. Over this period, the size of every injury in both the treatment and control groups progressively diminished. Upon scrutinizing the wound pictures closely, it was evident that the wound closure rate in wounds that were treated was noteworthy ($P < 0.05$). compared to injuries that were left untreated during the investigation. In contrast to the control group, the treatment group's data indicated that the garlic effect had either significantly enhanced wound contraction or significantly reduced wound size. Collagen and elastin synthesis, revascularization, wound contraction, a variety of vascular and cellular alterations, proliferation of epithelial and fibroblast cells, and collagen and elastin accumulation are all necessary for wound healing .In addition, it is noteworthy that our analysis of the treated wound sections' histological results shows a high incidence of trophic regeneration, as well as anti-inflammatory and analgesic qualities—all of which are essential for the healing process to be completed (Behm, B *et al* (2011) . In the control wound sections, mature granulation tissue, myofibroblasts, and new blood vessels were apparent; nevertheless, a few myofibroblasts were

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scattered throughout the fibrous connective tissue, which contained blood vessels that were obstructed. The findings of this study may be associated with the actions of garlic, which may be crucial in enhancing and hastening the healing of skin wounds. This result is in line with several previous research that have investigated the direct healing of tissue abnormalities with garlic. It has been proposed that the in vivo activity of garlic may cause particular tissue regeneration. (Demidova-Rice, *et al* (2007). conducted a research. Research on skin wounds treated with aged garlic extract revealed significant dose-dependent neovascularization and an increase in re-epithelialization (EJAZ, S, *et al* (2009). Rearranging ground collagen fibers was shown to be a crucial factor in this study's tissue healing and wound contraction, processes that may eventually affect the scars' quality. Endothelial cells can travel through the extracellular matrix during angiogenesis, which acts as a storehouse for growth factors and signals (Li, J. *et al* (2003). Unknown is the mechanism underlying tissue contraction during wound healing. Two theories are proposed to explain this process. According to the first idea, myofibroblasts produce the contractile forces, which are then transferred to neighboring cells and the connective tissue. According to this theory, the myofibroblasts would constrict the tissue by acting as a multicellular unit (Ottl, J *et al.* (1990). According to the second idea, fibroblast activity causes the collagen fibers to reorganize, which results in the contraction. Collagen fibers would realign into bigger bundles and compress as a result of the displacement stresses these cells in the connective tissue produced (Ehrlich, H. and Krummel, T.(1990) Berry, *et al.* (1998). All things considered, several cell types, extracellular matrix proteins, and mediators including growth factors and cytokines all have an influence on the intricate process of wound healing. When it came to wound healing, the use of 15 percent garlic extract produced the greatest and most improved outcomes. The clinical, macroscopic, and histological results, which demonstrated a significant difference ($P < 0.05$) between the garlic group and the control groups, clearly demonstrate this. Furthermore, by using 15% garlic extract topically, we may enhance and reinforce the skin wound therapy strategy.

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