Original
ArticleGarlic extract and phonophoresis in wound healing: Histological
and immunohistochemical studyOriginal
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ABSTRACT

Background: Garlic extract can be used alongside conventional antibiotics to fight agents of nosocomial infections, prevalent in hospitals. Phonophoresis is the use of therapeutic ultrasound to increase the percutaneous absorption of pharmacologic agents.

Aim of the work: To compare between the use of garlic extract with and without phonophoresis in wound healing in male albino rats.

Material & methods: Twenty four male albino rats were classified into 4 equal groups. Group I included 6 rats used as the control group. Group II; 6 rats subjected to wound injury; of them 3 were sacrificed after 2weeks (subgroup-IIa) and the other 3 rats were sacrificed after 3 weeks (subgroup IIb). Group III (Garlic-extract treated) included 6 rats subjected to wound injury and received garlic extract gel daily. Of them, 3 rats and were sacrificed after 2weeks of treatment (subgroup-IIIa). The other 3 rats were sacrificed after 3 weeks of teatment (subgroup-IIIb). Group IV (Garlic extract /Phonophoresis, both) 6 rats subjected to wound injury and received garlic extract gel daily together with phonophoresis 3 times/week. Of which, 3 rats sacrificed after 2 weeks (subgroup-IVa) and 3 rats were sacrificed after 3 weeks (subgroup-IVa) and 3 rats were sacrificed after 3 weeks (subgroup-IVa). The wound surface areas were measured. Skin sections were processed for histological, immunohistochemical and morphometric studies.

Results: Examination of skin sections in the wound group (group II) showed wide separation of edges and few hair follicles with reduced thickness and desquamated keratin. In group III and group IV, there was marked regeneration of the epidermis and dermis. With significant increase in hair follicles number in group IV compared to group III. Multiple immunostained CD44 +ve cells were detected in group IV.

Conclusion: Both garlic and phonophoresis helped wound healing but Garlic extract/phonophoresis combination revealed faster and better (i.e., more complete wound) healing compared to garlic extract application without phonophoresis.

Key Words: Garlic extract, phonophoresis, wound

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INTRODUCTION

Keratinocytes within the basal layer maintain stem cell-like characteristics and play an important role in the renewal and regeneration of damaged skin. During the formation of the stratum corneum, differentiated keratinocytes in the stratum granulosum, undergo cell death and release keratins. Although this layer contains no living cells, this layer is enzymatically active. Enzymes such as transglutaminase 1 and 3 cross link many of these proteins to protect the skin from mechanical insults in this layer, whereas various lipids give the skin its waterproof property^[1].

Wound healing is essential to defend the body against foreign substances such as microorganisms. The rapid migration of keratinocytes toward the injured area of the skin is important for the re-epithelialization of the skin. To support this process, a variety of growth factors such as epidermis growth factor (EGF) and fibroblast growth factor (FGF) are released from fibroblasts, platelets, mesenchymal cells and keratinocytes at the wounded skin area to facilitate keratinocyte migration^[2]. Wound healing proceeds via three overlapping phases: inflammatory phase with hemostasis and inflammation; proliferative phase with angiogenesis, collagen deposition and remodeling phase with connective tissue deposition. However, extended

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secretion of pro-inflammatory cytokines, such as tumor necrosis factor (TNF) or interleukin (IL)-1, prolongs the inflammatory phase resulting in chronic wounds or hypertrophic scar formation^[3].

Allicin, the active substance of garlic, exerts a broad spectrum of pharmacological activities and is considered to have potential therapeutic applications. The allicin may be useful in reducing oxidative stress, inflammation, vascular dysfunction. Many health benefits have been attributed to garlic, such as the treatment of arthralgia, leprosy, and epilepsy. It also showed antimicrobial effects again many viruses, bacteria, fungi and parasites^[4].

Numerous studies have demonstrated that garlic extract (GE) has strong anti-oxidant activity. Little is known regarding the anti-inflammatory activity of GE. In addition, fresh row GE was found to reduce PGE2, NO, IL-6, IL-1 β , GE might be helpful for the treatment of diseases mediated predominantly by reactive oxygen species^[5].

Garlic is constituted chemically of Allicin (released when crushed) an amino acid which gives Garlic its strong odor and is responsible for the powerful anti-bacterial actions. It is used in cases of acne, cutaneous eruptions, abscesses and wounds^[6].

Phonophoresis is a technique by which therapeutic ultrasound is used to introduce pharmacologic agents^[7]. Over the past 5 years, several studies showed that ultrasound, is able to kill bacteria by activating the sonosensitizers to produce reactive oxygen species, which are toxic to microbes. This work may open up the potential for the development of a novel form of ultrasound-mediated antimicrobial therapy. It is considered a future prospect for viable antimicrobial regime^[8].

Mesenchymal stem cells (MSCs) have been investigated as a clinical therapy to promote tissue repair. The researchers evaluated the relative contribution of grafted human MSCs and host stem/progenitor cells in wound healing. Grafted human MSCs improved healing in impaired healing animals by producing significant elevation of certain signals and increased the number of pre-existing host MSCs recruited to the wound bed^[9].

Cell replacement using stem cells & stem cell secretory factors showed beneficial effects in wound healing by reducing tissue damage and augmentation of endogenous repair through dermal fibroblast proliferation, migration and extracellular matrix production^[10].

The objective of this study was to compare between the use of Garlic extract with and without phonophoresis in providing faster wound healing in rats.

PATIENTS AND METHODS

The study was performed at the Animal House of Kasr El Aini, Faculty of Medicine, Cairo University, according to the guide for the care and use of laboratory animals.

Animal Design:

Twenty four male albino rats weighing 200300-g were housed in a temperature and light-controlled room, with free access to food and water. The animals were classified into the following groups, which were kept in separate cages:

Control group (group I): Included 6 rats, treated with KY gel film, 1 rat sacrificed with the rats of each corresponding treated subgroup.

Wound group (group II): Included 6 rats, rats were exposed to wound injury on the dorsal back and were divided into 2 subgroups:

Subgroup IIa 3 rats sacrificed after 2 weeks.

Subgroup IIb 3 rats sacrificed after 3 weeks following wound injury. To asses spontaneous recovery

Garlic extract group (Group III): Included 6 rats subjected to wound injury as in group II then garlic extract gel film of 1 mm thick was applied daily. 3 rats were sacrificed 2 weeks (subgroup IIIa) and 3 rats were sacrificed 3 weeks (subgroup IIIb) after therapy.

Garlic extract/phonophoresis group (Group IV): Included 6 rats subjected to wound injury and garlic extract gel was applied daily then phonophoresis were applied 3 times per week. 3 rats were sacrificed 2 weeks (subgroup IVa) and 3 rats were sacrificed 3 weeks (subgroup IVb) after therapy.

Garlic extract group (Group III): Included 6 rats subjected to wound injury as in group II then garlic extract gel film of 1 mm thick was applied daily. 3 rats were sacrificed 2 weeks (subgroup IIIa) and 3 rats were sacrificed 3 weeks (subgroup IIIb) after therapy.

Garlic extract/phonophoresis group (Group IV): Included 6 rats subjected to wound injury and garlic extract gel was applied daily then phonophoresis were applied 3 times per week. 3 rats were sacrificed 2 weeks (subgroup IVa) and 3 rats were sacrificed 3 weeks (subgroup IVb) after therapy.

Procedure of wound induction

In preparation for surgery, the rats were fasted 12 hours for food while water was withheld 3 hours only before the operation. Immediately before the surgery the hair was removed from the site chosen by the veterinarian, at the dorsal back on the right side of the spine (fixed in all animals) using hair removal cream, the remaining hair was short cut using hair scissor. To induce anesthesia, ketamine hydrochloride (Ketalar), (Parke Davis Barcelona, Spain) (35 mg/kg) was injected into the gluteus maximus muscle of the animal. Using aseptic techniques, the veterinarian produced a 1cm diameter circular skin incision along the back below the shoulder and above the thigh on the right side of the vertebral column about 5 cm away from the vertebral column following shaving of hair (Fig.1). Betadine was applied to the wound site and rinsing with normal saline was performed at the site of injury immediately after the operation. Then aseptic dressing was applied on the wound^[11].

Therapeutic equipments

The Sonopuls 190 ultrasonic unit, Allicin gel treatment protocols were achieved by using the ultrasound therapy unit.

1- Sonopuls 190 (Ultrasonic Therapy Unit)

The sonopuls 190 (Enraf Nonius)^[12] is a single channel Ultra Sound (US) unit. The compact design and the simple operation displays make the Sonopuls 190 very useful as a standalone unit. The ultrasound treatment head has a fixed connection to the unit and cannot be connected to other units without calibration. The sonopuls 190 has been designed and manufactured to ensure the highest level of safety: the unit itself fully complies with the International Electrotechnical Commission (IEC) 6011- and IEC 601-2-1 standards. The power supply complies with the IEC 950standards. The power supply unit is a main supply adapter which has a voltage of 100240- volts alternating current vac (VAC), the main frequency is 5060- Hz, and the supply adapter output voltage is 15 volts direct current (VDC), and its current has a minimum of 2 A. The ultrasound frequency is 1 MHz $\pm 0.2\%$, the output mode is continuous with a pulse frequency of 100 Hz and 100% duty cycle. The intensity is 02-W/cm² in a continuous mode. The output mode can be pulsed with a pulse frequency of 100 Hz, with duty cycles varying between 5 and 100% (5, 10, 20, 50, 100). The intensity varies between 03- W/cm² in duty cycles of (5, 10, 20, 50) %, while it varies between 02- W/cm² with a duty cycle of 80%.

2-Garlic-extract gel

The garlic extract gel was prepared in the laboratory of the Faculty of Pharmacy, Cairo University. The garlic extract was prepared through buying the garlic from a local vegetable market. Then the garlic bulbs were peeled and ground to form a paste in 1 gram quantity. The paste then was dissolved in 100 ml distilled water in sterile tube. The solution was centrifuged at 6000 rpm for 20 minutes at room temperature. The pellet was discarded supernatant was diluted 10 times with KY gel to get 1% of garlic extract $(GE)^{[13]}$. Allicin is considered the main active constituent of $GE^{[14]}$. The gel was taken from the jar by the tip of a sterile stick and a film of about 1 mm thick was applied to the wound surface.

Treatment procedure:

Rats were treated at the injured area according to a treatment regimen of 3 sessions per week during the $study^{[15]}$ and $^{[16]}$.

o Before treatment the skin was cleaned with saline.

o The treatment protocol was applied to both groups from the first day post operative and continued throughout the study at a frequency of 3 sessions per week for 3 weeks.

o Garlic extract gel was applied to group III & IV, in group IV only, 20% duty cycle of ultrasound with an intensity of 1.5W/cm² of ultrasound was applied on the wound surface with a frequency of 1MHz for 5 minutes^[17].

o Only one person was responsible to provide treatment for all animals to standardize the handling process.

o The rats were anesthetized for the application of the treatment, and were allowed to lie on their left side exposing the wound on their right side.

o A thin film of garlic gel (1mm thickness) was applied followed by ultrasound therapy in small and slow circular movements around the wound edges touching the wound area (Fig. 2).

Wound surface area measurements:

A picture of the wounds was taken at a 15 cm distance away from the wound with a Sony digital camera 12 Mega Pixels and then the picture was processed by the image J software for exact dimensions. The wound was traced with the polygon selection in the tools bar that the image J measured afterwards. Another picture of a square of area measuring 1cm² was taken as a reference to calibrate the wound measurement in cm² when needed (Fig. 3) and it was equal 102704 pixels. The pictures of the wounds were taken before the beginning of the treatment and another picture was taken at the end of 2nd week and 3rd week of treatment for comparison^[18].

The animals were sacrificed by cervical dislocation^[19]. Skin specimens were removed from the back (including raw area and a margin of 5 mm normal skin around) and fixed in 10% formol saline for 48 hours in control and experimental groups. Paraffin blocks were prepared and 5µm thick sections were subjected to the following studies:

1- Hematoxylin and eosin^[20]

2-Immunohistochemical (IHC) Study:

CD44 (IW-PA1021) antibodies were used to detect the endogenous mesenchymal stem cells^[21]. 0.1 ml primary antibody rabbit polyclonal Ab were applied to sections

for 60 minutes. Counter stain the slides with Mayer-Hematoxylin for 13- min. Tonsil used as positive specimens which gave a brown coloration. Cellular localization is the cell membrane.

Morphometric Study

Using Leica Qwin 500 LTD computer assisted image analysis system (Cambridge, United Kingdom), assessment of the distance between the margins of epidermis and dermis in H&E stained sections was performed by interactive measurements menu. The number of hair follicles was counted in H&E stained sections in 10 low power fields (LPF) from each group. The area % of CD44 immunoexpression was done using binary mode. The measurements were done in 10 high power fields (HPF) in experimental groups^[22].

Statistical analysis

Quantitative data were summarized as means and standard deviations and compared using one-way analysisof-variance (ANOVA). Any significant ANOVA was followed by Bonferroni post-hoc test to detect which pairs of groups caused the significant difference. P-values <0.05 were considered statistically significant. Calculations were made on SPSS 9.0 software^[23].

RESULTS

Wound surface area measurements

There was a statistically significant difference between group II wound surface area (2- and 3-weeks post-treatment) compared to group III and group IV. There was no wound by the 3rd week. There was no statistically significant difference between the surface areas of the wounds in the 2 groups (group II and group IV) along the various durations, as the P-value level was more than 0.05 (Fig.3).

Histological results

Examination of Hematoxylin and Eosin (H&E) stained skin sections in control group showed epidermis and dermis containing multiple hair follicles (Fig. 4). At higher magnification, the stratified squamous epithelium of the epidermis and the connective tissue of dermis were seen. Stratified squamous epithelium of hair follicles surrounding keratin was noticed (Fig. 5).

Examination of wound group (subgroup IIa) showed wide spacing of edges, desquamated keratin. Dislodged sebaceous glands not associated with hair follicles were noticed (Fig. 6). At higher magnification, separation of dermal connective tissue, reduced thickness of hair follicle layers with desquamated keratin were seen (Fig. 7). While in subgroup IIb, sections showed disrupted epidermis and dermis with wide spacing of edges and tissue debris in between (Fig. 8).

Skin sections of group III (subgroup IIIa) revealed discontinuous epithelium and desquamated keratin. Hair follicle with reduced layers was noticed (Fig. 9). In subgroup IIIb showed thin epidermal layer at the site of fusion (Fig. 10).

Skin section of group IV (subgroup IVa) showed disrupted epidermis and dermis with less spacing of edges, disrupted keratin, disrupted epithelial lining of a follicle and minimal separation of CT fibers (Fig. 11). Subgroup IVb showed a hair with developed keratin and developed epithelial lining of a hair follicle (Fig. 12). Other fields showed continuous surface epithelium with thin horny layer. A deep follicle with traces of keratin and multiple deep developing follicles with keratin are seen. Superficial follicle with reduced thickness, a congested capillary was noticed (Fig. 13).

Immunohistochemical results

Examination of skin section of group I (control group) showed –ve CD44 cells (Fig. 14). In group II (subgroups IIa and IIb) showed few +ve CD44 cells (Fig. 15 & 16). Skin section of group III (subgroups IIIa and IIIb) showed some +ve CD44 cells in the basal layer of epidermis, dermis and in hair follicles (Fig. 17 & 18). Skin section of group IV (subgroups IVa & IVb) showed multiple +ve CD44 cells in the basal layer of epidermis, dermis and hair follicles (Fig. 19 & 20).

Morphometric results

Distance between disrupted edges of epidermis:

A significant increase (P < 0.05) in the mean distance between disrupted edges of epidermis was recorded in subgroups IIa compared to other subgroups (Table 2).

Number of hair follicles:

A significant increase (P < 0.05) in the mean number of hair follicles in the control group and subgroup IVa & IVb compared to subgroups IIa, IIb, IIIa and IIIb (Table 2).

Area % of CD44 immunoexpression:

A significant increase (P < 0.05) was reported in subgroup IVa and IVb compared to subgroup IIa & IIb (Table 2).

Variable	Group II	Group III	Group IV
2 weeks Post-treatment	95602.2±9779.60*	802.8±727.42	759.6±747.10
3 weeks Post treatment	30703.0±3740.84*	0±0	0 ± 0

Table 1: Comparison between wound measurements in experimental groups

* Significant increase of healing in group II compared to group III and group IV (2 and 3-weeks post treatment). P-value was less than 0.05.

Table (2): Mean distance between disrupted edges of epidermis and area % of CD44 immunoexpression in experimental subgroups (±SD).

Group	Mean distance	Number of hair follicles	Mean area % of CD44
Control group	-	32±4#	-
subgroup IIa	4350.98±651.71*	10±21	3.01±0.44
subgroup IIb	1974.19±331.23	8±13	3.28±0.76
subgroup IIIa	1632.21±455.22	12±30	6.51±37
subgroup IIIb	0 ±0	14±34	5.43±61
subgroup IVa	1014.08±131.53	28±22#	8.96±1.65**
subgroup IVb	0 ±0	30±51#	8.20±1.99**

* Significant increase (P<0.05) compared to all other subgroups ♯ Significant increase (P<0.05) compared to subgroups IIa, IIb, IIIa & IIIb.

** Significant increase (P<0.05) compared to subgroups IIa & IIb



Fig. 1: Wound induced pretreatment.



Fig. 2: Application of phonophoresis.

GARLIC/PHONOPHORESIS IN WOUND



Fig. 3: Tracing and measuring the wound size with image J.



Fig. 4: Skin section of control (G1) showing epidermis (E) and dermis (D) containig multiple hair follicles (F) (H&E, x100)



Fig. 5: Skin section of control (GI) showing stratified squamous epithelium (SE) of epidermis, connective tissue (CT) of dermis. Note stratified squamous epithelium (SE) of hair follicle surrounding keratin (K) (H&E, x200)



Fig. 6: Skin section of wound group (subgroup IIa) showing wide spacing of edges (arrowheads), desquamated keratin (bifid arrows). Note dislodged sebaceous gland not associated with hair follicles (H&E x100)



Fig. 7: Skin section of wound group (subgroup IIa) showing separation of dermal connective tissue (wavy arrow), reduced thickness of hair follicle layers (arrowhead) with desquamated keratin (arrow) (H&E, x200)



Fig. 8: Skin section of wound group (subgroup IIb) showing disrupted epidermis and dermis with wide spacing of edges (arrows) and tissue debris inbetween (arrowhead) (H&E, x200)



Fig. 12: Skin section of garlic extract/phonophoresis group (subgroup IVb) showing a hair with developing keratin (arrow) and developing epithelial lining of a hair follicle (arrowhead) (H&E x100)



Fig. 13: Skin section of group garlic extract/phonophoresis (subgroup IVb) showing continuous surface epithelium with thin horny layer (bifid arrow). A deep follicle with traces of keratin (arrowhead) and multiple deep developing follicles with keratin (wavy arrows) are seen. Superficial follicle with reduced thickness (arrow), Note a congested capillary (c) (H&E x200)



Fig. 9: Skin section of garlic group (subgroup IIIa) showing discontinuous epithelium (arrow) and desquamated keratin (square). Note hair follicle with reduced layers (arrowhead) (H&E, x100)



Fig. 10: Skin section of garlic group (subgroup IIIb) showing thin epidermal layer at the site of fusion (arrow)



Fig. 11: Skin section of garlic extract/phonophoresis group (subgroup IVa) showing disrupted epidermis and dermis with less spacing of edges (arrows), disrupted keratin (square), disrupted epithelial lining of a follicle (arrowhead) and minimal separation of CT fibers (star) (H&E, x200)

GARLIC/PHONOPHORESIS IN WOUND



Fig. 14: Skin section of group I showing –ve immunostaining (CD44 immunostaining, x200)



Fig.15: Skin section of subgroup IIa showing +ve immunostaining (CD44 immunostaining, x200)



Fig. 16: Skin section of subgroup IIb showing some positive immunostained spindle cells (arrows) in the epidermis and dermis (CD44 immunostaining, x200)



Fig. 17: Skin section of subgroup IIIa showing multiple positive immunostained cells (arrows) in the basal layer of epidermis, dermis and few hair follicles (CD44 immunostaining, x200)



Fig. 18: Skin section of subgroup IIIb showing some positive immunostained cells (arrows) in multiple hair follicles (CD44 immunostaining, x200)



Fig. 19: Skin section of subgroup IVa showing multiple positive immunostained cells (arrows) in the basal layer of epidermis, dermis and few hair follicles (CD44 immunostaining, x200)



Fig. 20: Skin section of subgroup IVb showing multiple positive immunostained cells (arrows) in hair follicles (CD44 immunostaining, x200)

DISCUSSION

The current study demonstrated modulating effect of garlic extract gel and garlic extract/phonophoresis on induced wound injury in albino rat. This was evidenced by wound surface area measurements, histological, immunohistochemical and morphometric studies.

Examination of group II revealed significant increase in wound surface area compared to group III and IV. Histological examination recruited in (subgroup IIa) a significant increase in the mean distance between disrupted edges of epidermis compared to other subgroups with separation of dermal connective tissue and reduced thickness of hair follicle layers with desquamated keratin. While subgroup IIb showed disrupted epidermis and dermis with wide spacing of edges and tissue debris. These finding may be due to inflammatory reaction induced by wound injury. Noteworthy, delayed cutaneous wound healing usually results in local infection and may lead to chronic, non healing wounds^[18].

Examination of skin sections in garlic group (subgroup IIIa) showed discontinuous epithelium, desquamated keratin and hair follicle with reduced layers. While subgroup IIIb showed thin epidermal layer at the site of fusion. These changes indicated partial improvement in the wound area due to garlic gel application. It was proved that allicin has antioxidant, anti-hypertrophic and anti-apoptotic properties^[24]). It was stated that there may be a role for garlic extract in resolving inflammation by inducing the apoptosis of responding immune cells, as the need for them is decreased^[25]. There was an evidence for effect of garlic extract (allicin) by shortening the inflammatory phase and maturation of collagen bundles in treated wounds^[26].

Garlic extract/phonophoresis (subgroup IVa) showed epidermis and dermis with less spacing of edges, disrupted keratin and minimal separation of CT fibers. While in subgroup IVb showed continuous epidermis with thin horny layer. Multiple deep developing follicles with keratin were seen. Superficial follicle with developed keratin and developed hair was seen with significant increase in the mean number of hair follicles in subgroup IVa & IVb compared to other experimental group. These changes proved evident regeneration. It was recorded that the garlic-derived chemo-preventive agent allicin is potent radical-trapping antioxidants in lipid bilayers^[27]. There was reduced production of pro-inflammatory cytokines and nitric oxide (NO) in response to allicin treatment^[28].

Low intensity ultrasound (US) induced enhancement of plasma membrane permeability and is considered a promising tool for delivering exogenous vectors at the specific biological site in a safe and efficient way^[29]. On the other hand, increasing ultrasound intensity induced high reactive oxygen species production^[30]. Microfocused ultrasound was recently introduced as a novel energy modality for transcutaneous heat delivery that reaches the deeper sub-dermal connective tissue at consistent programmed depths. Outcomes of low and high frequency ultrasound therapy were better than standard care alone^[31].

Examination of skin section in group II showed few +ve CD44 cells. In group III, sections showed some +ve CD44 cells in the basal layer of epidermis, dermis and in hair follicles. Significant increase in the mean area% of CD44 was recorded in group IV compared to group II. This may be contributed to endogenous stem cells activation. This may be explained by progenitor cells activation that was present in the CT of dermis or hypodermis or it may be stimulated from other endogenous sources. It was determined the proliferative capacities of bone marrow-derived MSCs^[32]. Their isolation was based on morphology and expression of CD44^[33]. SCs accelerate wound closure with an increased level of reepithelialization, neovascularization, and regeneration of skin appendages. Concomitantly, it was proved that postnatal stem/progenitor cells hold great promise to

enhance repair of damaged tissues^[34]. Many of these cells are retrieved from bone marrow or adipose tissue. They were described as fibroblastic cells, plastic-adherent and exhibit a surface marker profile positive for CD73, CD44, and CD90. They were defined by the International Society for Cellular Therapy as MSCs^[35].

CONCLUSION

Garlic extract/phonophoresis induced sterile, fast and complete wound healing compared to garlic extract application without phonophoresis. The definite therapeutic effect was confirmed by morphometric results regarding the distance between epidermal edges and number of developing hair follicles which related to activation of endogenous mesenchymal stem cells.

CONFLICT OF INTEREST

There is no conflict of interest to declare.

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الملخص العربى

مقارنة تأثير مستخلص الثوم معه مصحوباً بالموجات فوق الصوتية في التئام الجروح: دراسة هستولوجية واميونو هستوكيميائية

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المقدمة: يمكن استخدام خلاصة مسحوق الثوم لمقاومة مجموعة من العدوي المنتشرة في المستشفيات. استحدثت تقنية الموجات فوق الصوتية كعامل علاجي و دوائي.

هدف البحث: المقارنة بين استخدام مستخلص الثوم فقط ومستخلص الثوم مصحوبا بالموجات فوق الصوتية للحصول علي النئام أسرع للجروح في الجرذان.

مواد وأساليب البحث: تم تقسيم 24 جرذ من الذكور البيضاء الي اربع مجموعات متساوية. المجموعة الأولي (المجموعة الضابطة): اشتملت علي 6 جرذان. والمجموعة الثانية (6 جرذان) تم تعريضها لجرح ثم تمت التضحية ب 3 جرذان بعد اسبوعين (مجموعة ثانية أ) وتمت التضحية ب 3 جرذان بعد اسبوعين (مجموعة ثانية ب). والمجموعة الثالثة (مجموعة مستخلص الثوم) اشتملت علي 6 جرذان تم تعريضهم للجرح واستقبلوا العلاج بمستخلص الثوم بدون استخدام الموجات فوق الصوتية. 3 جرذان منهم تما تم تعريضها لجرح ثم تمت التضحية ب 3 جرذان بعد اسبوعين المتملت علي 6 جرذان تم تعريضهم للجرح واستقبلوا العلاج بمستخلص الثوم بدون استخدام الموجات فوق الصوتية. 3 جرذان المتملت علي 6 جرذان تم تعريضهم للجرح واستقبلوا العلاج بمستخلص الثوم بدون استخدام الموجات فوق الصوتية. 3 جرذان منهم تم التضحية بهم بعد اسبوعين (المجموعة الثالثة أ) و 3 جرذان تم التضحية بهم بعد 3 سابيع(المجموعة الثالثة ب). والمجموعة الثالثة ب).و المجموعة الثالثة ب).و المجموعة الثالثة ب) و 3 جرذان تم التضحية بهم بعد 3 سابيع(المجموعة الثالثة أ) و 3 جرذان تم التضحية بهم بعد 3 سابيع(المجموعة الثالثة أ) و 3 جرذان تم التضحية بهم بعد 3 سابيع(المجموعة الثالثة ب).و المجموعة الثالثة ب).و المجموعة الثالثة ب) و 3 جرذان تم التضحية بهم بعد 3 سابيع(المجموعة الثالثة با).و 1 منهم تم التضحية بهم بعد 3 سابيع(المجموعة الثالثة أ) و 3 جرذان تم التضحية بهم بعد 3 سابيع(المجموعة الثالثة با).و المجموعة الرابعة (مجموعة الرابعة (مجموعة الثالثة با).و 1 جرذان منهم تم التضحية بهم بعد 1 سبوعين (المجموعة الموجبة فوق الصوتية) المتملت علي 6 جرذان تم تعريضهم لجرح واستقبلوا العلاج بمستخلص الثوم عن طريق استخدام الموجات فوق الصوتية. 3 جرذان منهم تم التضحية بهم بعد اسبوعين (المجموعة الرابعة أ) و 3 جرذان منهم تم التضحية بهم بعد 1 سبوعين (المجموعة الرابعة بال وعمل قلياس لموجبة). وتم عمل قياس لمساحة الجرح وت دراسة قطاعات الرابعة أ) و 3 جرذان منهم تم التضحية بهم بعد والم وي در المجموعة الرابعة بال وي و مرذان تم الجرح.وت دراسة قطاعات الحربة أي و 3 جرذان تم المواحي لي والمولي والعالي الموجبة ما مولي الحربة مولي الموجبة موقا الحربة والمولي الموجبة مولي الموجبة مولي الموجبة مولي الموجبة مولي الموجبة والموبة مولية ما مولي ما مولي ما مولي مولي ما مولي و مردان مول

النتائج: أظهرت نتائج فحص قطاعات الجلد فى مجموعة الجرح وجود مسافات واسعة بين أطراف الجرح مع وجود عدد قليل من بصيلات الشعر وأيضا انخفاض في سمك طبقة الجلد مع انفصال لطبقة الكيراتين. بينما في المجموعة الثالثة والرابعة ظهر تحسن ملحوظ في سمك طبقة الجلد. مع وجود زيادة ذات دلالة احصائية في عدد بصيلات الشعر في المجموعة الرابعة مقارنة بالمجموعة الثالثة. وتم ملاحظة عدد كبير من الخلايا المصبوغة مناعيا بالسي دى 44 في المجموعة الرابعة.

الاستنتاج: يساعد استخدام كلا من مستخلص الثوم ومستخلص الثوم مع الموجات فوق الصوتية في التئام جروح ذكر الجرذ الأبيض. ولكن استخدام مستخلص الثوم مصحوبا بالموجات فوق الصوتية يؤدي الي التئام أسرع وتام للجرح عنه بدون الموجات فوق الصوتية.