Original Article

INFLUENCE OF CONTACT ULTRASONIC WITH DIFFERENT POWER DENSITIES ON FULL-THICKNESS WOUNDS HEALING: AN EXPERIMENTAL STUDY

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ABSTRACT

Effect of ultrasound power density on wound-healing process in rats was investigated. Forty five adult *albino* male albino rats were divided into 3 equal groups (15 rats in each group); one was used as a control group while the others were subjected to ultrasound treatment of power density of 0.5 W/cm^2 and 1 W/cm^2. All rats were anesthetized by inhalation (diethyl ether) and a 2 x 2.5 cm area wound was made on the dorsum. Rats in groups **A** and **B** were treated with pulsed therapeutic ultrasound for 10 min, 3 times a week at a frequency of 1 MHz and at a power density of 0.5 W/cm^2 and 1 W/cm^2, respectively, while rats in group C received sham ultrasound. Subsequently, the wound was captured by digital camera and was measured using transparent film, metric graph paper and flexible ruler 3 times a week until closure was complete**.** Significant reduction of the wound area, and linear dimensions in all three groups of rats was observed. After 14 days of treatment, wound area reduction as well as wound dimensions reduction were more pronounced in group A and to a lesser extent in group B in comparison to group C. Results indicated that ultrasound at 0.5 W/cm^2 and 1 W/cm^2 caused positive changes in the healing process with ultrasound at 0.5 W/cm^2 being more effective.

KEYWORDS: Full thickness Wounds, Rats, Ultrasonic, Power Density, Physiotherapy.

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INTRODUCTION

Wound is a breach formed in the normal continuum of the cellular and molecular structure of the body, thereby creating a disruption in the cellular, anatomic and as well as in their functional continuity. Wound healing or wound repair is an intricate process in which the skin or organ or tissue repairs itself after injury [1]. Wound can be healed as spontaneous process in the organism through a cascade of events, which starts by switching on various chemical signals in the body. While partial thickness wound heals by mere epithelialization, the healing of full thickness wound which extends through the entire dermis involves more complex well-regulated biological events. The healing process begins with the clotting of blood and is completed with remodeling of the cellular layers of the skin. The whole process can broadly be classified into 5 overlapping phases, namely, inflammation, granular tissue formation, re-epithelialization, matrix production and remodeling. [2]

Tissue injury results in local vascular injury. The ensuing bleeding floods the wound with mediators of the coagulation cascade. Factors are released to attract platelets to the site of injury. These include mediators such as platelet-derived growth factor and transforming growth factor beta. Macrophages are attracted to the site of injury by these factors, debride local necrotic tissue and orchestrate the wound healing process by releasing cytokines such as interleu-

kins and other factors [3]. They also stimulate, via these cytokines, fibroblasts to begin collagen production and smooth muscle and endothelial cells to proliferate for angiogenesis. As the collagen matrix and new vessels bridge the wound, the surface undergoes epithelialization. These processes continue until contact inhibition causes it to stop. Throughout this healing and for many months after the wound has closed, collagenase breaks down the newly formed collagen while new collagen is deposited. This balanced process of collagen production and collagen destruction results in wound remodeling.[4]

These mechanisms of wound repair occur to different degrees during the dirrerent types of healing. Healing by secondary intention occurs in full thickness wounds or wounds involving the epidermis and whole dermis layer. Finally, partial thickness wounds or wounds involving the epidermis and part of the dermis heal by epithelialization[(5)]. Open wounds have been treated with medicines and a range of natural and synthetic materials in an attempt to speed healing. Despite considerable laboratory and clinical study no single therapy has proved beneficial for all types of wounds and problems related to wound healing are still the cause of significant morbidity and mortality. [6]

Studies on wound healing have increased our knowledge and understanding of wound, which constitute an important clinical problem in rehabilitation medicine. Most studies concerning wound healing focus on accelerating wound and soft tissue healing, obtaining normal wound breaking strength, and preventing keloid and scar formation. Recently, some researchers found that some physical methods as constructive, adjunctive niches in facilitating and accelerating wound healing, and also improving scar quality, they include; therapeutic ultrasound, laser treatments, hydrotherapy, negative pressure therapy, hyperbaric oxygen and electrostimulation [7]. Therapeutic ultrasound has been widely used over the past 50 years to treat many musculoskeletal complaints, including tendon injuries, pressure sores, venous ulcers, poor wound healing, lateral epicondylitis, herpeszoster, muscle damage, Dupuytren's contractures, and others. Since the 1960s. [8]

Ultrasound is defined as a mechanical vibration above the upper threshold of human hearing (>20 KHz. Therapeutic ultrasound has a frequency of 0.75–3.00 MHz, and most machines used to deliver it are set at a frequency of either 1 or 3 MHz. The absorption coefficient of ultrasound in soft tissue increases linearly with frequency, so using higher frequencies (say 3 rather than 1 MHz) reduces the penetration depth by about one-third (from 37 to 12 mm in skin**)**. Ultrasound can be produced in the form of continuous or pulsed waves. In the continuous mode, it is characterized by the production of biophysical and thermal effects, whereas in the pulsed mode it reduces the thermal effect due to the cyclical interruption of energy emission, while maintaining the biological effect. It has been suggested that the non-thermal effects of ultrasound, including cavitation and acoustic micromassage, are more important for the treatment of soft tissue lesions than the thermal effects.[9]

Although the exact mechanism underlying its clinical effects is not known, therapeutic US has been shown to have a variety of effects at a cellular level including angiogenesis, leukocyte adhesion, growth factor and collagen production, and increases in macrophage responsiveness, fibrinolysis and nitric oxide levels[10] . The type of ultrasound used is dependent on the target tissues (structure and depth) and the intended effect (i.e. heating the tissues or not). Tissues with higher protein content (e.g. ligament and tendon) are able to absorb ultrasound to a greater extent than those with a low protein content (e.g. blood and fat) [9]. Selection of ultrasound application parameters is based on the desired effect and the location and density of the tissue to be treated. These decisions are best made by the physician and the therapist experienced in performing therapeutic ultrasound.[11]

High power, high frequency ultrasound is defined as ultrasound of 0.5-10 MHz and up to1500W/cm2 while low power, low frequency ultrasound is defined as an ultrasound of 20- 120 kHz and 0.05-1.0 W/cm2). Low frequency/low intensity US is mainly reflected in the skin or wound surface. Only a small portion of the energy transmitted by the probe reaches deeper tissue

layers and the major effect is mechanical effect, which is the opposite for high frequency US that combine mostly thermal with mechanical effects[12]. Recently, low frequency ultrasound was tested and introduced to the market. The motivation of looking for alternative ultrasound parameters was due to the fact that application of high-frequency US in clinical medicine is limited due to tissue heating. Thus, using low-frequency US with less tissue heating, thereby acting as a "slow release" mechanism, may become the standard care in treating slow-to-heal lesions, skin ulcers and nonunion fractures. In addition it may be able to facilitate protein secretion and enzymatic reactions.[13]

The therapeutic range of ultrasound power density is between 0.1 and 3W/cm2. Different mechanisms responsible for the effect of ultrasound occur or dominate in the different bands of the range. This has an effect on the selection of optimum technical and application parameters of ultrasound used to enhance the healing of wounds. Following a thorough review of literature on the use of ultrasound for wound healing enhancement (power densities used are in the range 0.1–1W/cm2– spatial average–temporal average)[14]. So we decided to select two densities out from the recommended therapeutic range as the objective of our study was to determine which ultrasound power density (0.5W/cm2or 1W/ cm2) is more effective in reducing the area of full thickness wound in rats.

MATERIALS AND METHODS

A. Materials

Animals**:**

Forty five adult male albino rats approximately 4 months of age at study initiation, and weighed 200-250 gm obtained from animal house of Faculty of medicine, Umm Al Qura University. Rats were cared for in accordance with the Guide for the Care and Use of Experimental Animals of Umm Al-Qura University, KSA, and the entire experiment was carried out in accordance with the International Principles of Laboratory Animal Research. Rats were individually housed in stainless steel cages with wire-mesh flooring in a controlled environment at 23-25°C and 50% humidity with a 12 h artificial light cycle on a 12:12-h dark-light cycle (07.00-19.00 lights on). Food (#5322 Purina Certified Rat Ration, and available ad libitum for all. Animals were housed in solid bottomed cages; food and water were maintained on a pellet diet and tap water ad libitum during the entire period of the study (2 weeks).

Devices and Tools

Ultrasonic device**:** An ultrasound device (model Sonopuls 463, Enraf Nonius Co. The Netherlands) was used with a probe 1.9 cm^2 in diameter (Enraf-Nonius Co.).

Measuring instruments and tools: Transparent film, Metric graph paper, flexible ruler and digital camera, (Canon INC., Japan).

B. Methods:

A. Wound surgery: For all Forty five rats, local preparation of the dorsal skin was done; as dorsal skin of rats was shaved by electrical clipper, disinfected with 70% alcohol, after that, all rats were anesthetized by inhalation (diethyl ether). After anesthesia , the area for wound was measured (2x2.5 cm). A full-thickness excisional wound was performed. An area of wound 5 cm^2 in diameter (2x2.5 cm) was excised from the dorsal aspect of all rats. The same researcher performed all surgical procedures.

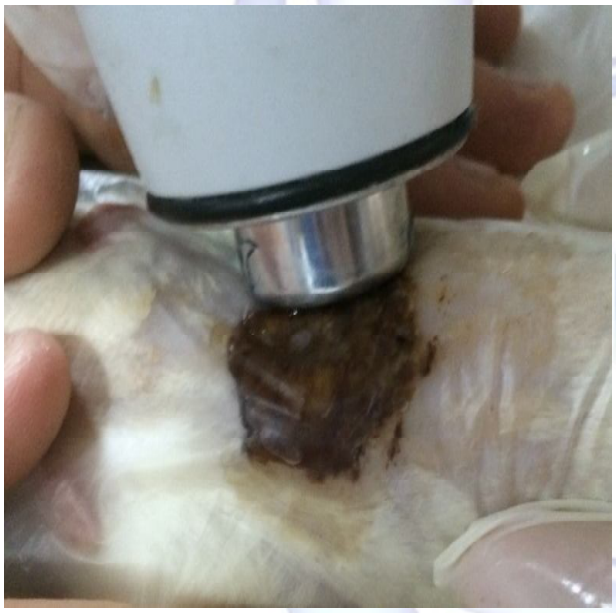
B: Treatment intervention: After wound surgery, rats were randomly divided into three groups: A, B and C. (15 animals in each group) and the treatment was started in each group within 2 hours of the surgical procedure.

The ultrasonic therapy treatment protocol include: Standard cleaning of wound by alcohol, Sterile coupling gel was put onto the wound and filled the wound cavity then Sterile plastic drape was applied to the wound and surrounding tissues to prevent any cross-contamination of the device. (The plastic drape should overlap wound margins by at least 5 cm).The sterile coupling gel was applied again on plastic drape and over wound area.[15]

Pulsed ultrasonic therapy (applicator 1.9 cm^2) was applied over plastic drape and moved within the wound boundaries with the following fixed parameters for both groups (A& B); pulsed duty cycle 20% (2 ms on, 8 ms off). Power density for group (A) was (1W/cm^2), while, power density for group (B) was (0.5 W/cm^2). In group C,

ultrasound treatment was applied with no current (sham method) in order to control any effect of; topical sonic gel and handling of ultrasonic. In all groups, moving of ultrasonic applicator was done for 10 minutes per day, day after day, three treatments per week and for 2 weeks. No preferred treatment of wound margins.(ultrasonic procedure shown in figure1**).**Gel and plastic drape were removed and, Wounds in all three groups were cleaned, dried and disinfected by povidone solution at the end of each session.

Fig. 1: Treatment procedure; rat grasping, contact ultrasonic applied to wound through sterile plastic drape.



C: Measurement:

The wound area was measured by placing a transparent tracing paper over the wound and tracing it out. The tracing paper was placed on 1 mm2 graph sheet, and traced out. The squares were counted and the areas were recorded, this shown in figure (2) (A&B). In each group, measurement of wound area was repeated three time; at (3rd, 7th and 14th days) of treatment.

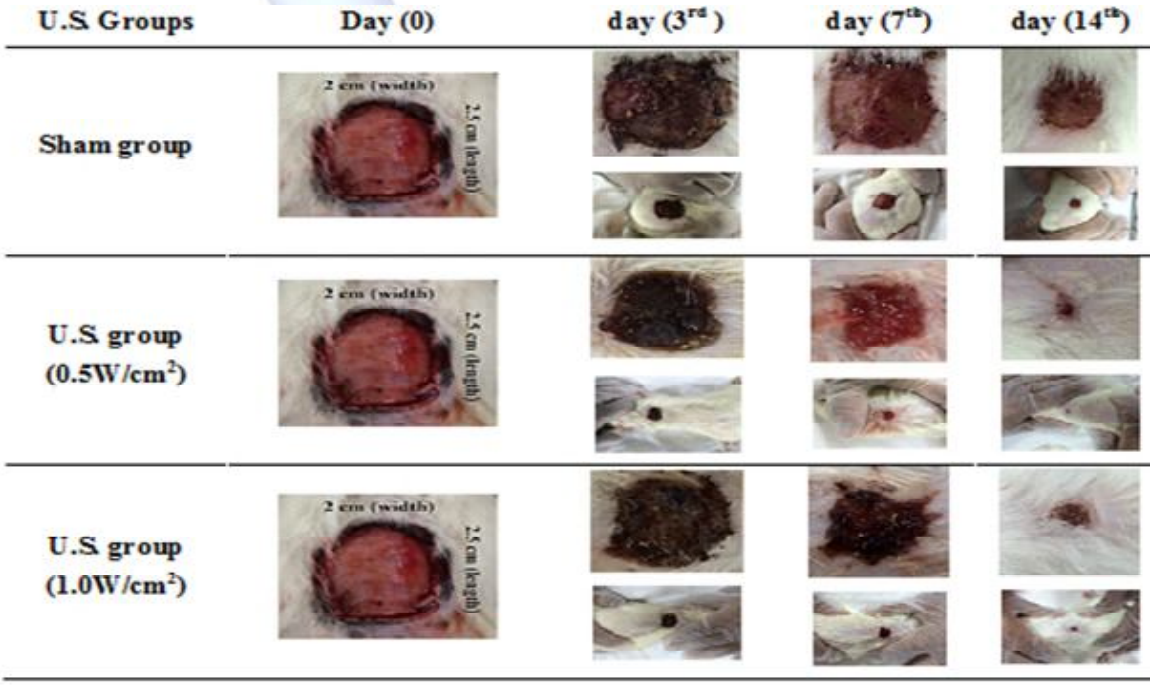
Flexible ruler was used also to measure the widest and longest parameters of each wound at each day (3rd, 7th and 14th days) of measurement in all groups, this shown in Figure (3).

Every wound area was captured by digital camera at each day (0 day, 3th, 7th and 14th days) of measurement in all groups, this shown in figure (4).

Fig. 2A, **2B** and **3:** Measurement of wound was done in triplicate (at 3rd, 7th and 14th days after treatment).

Figure 2A Figure 2B Figure 3

Fig. 4: Examples of digital camera photos for wound surface areas for three groups in the four phases of measurements.



The results were analyzed using repeated measures ANOVA to compare mean values within each group and one way ANOVA to compare mean values between groups and p value less than 0.05 is significant point

RESULTS AND TABLES

All forty five Rats were homogeneous in terms of characteristics; all rats were adult male albino rats approximately 4 months of age, and weighed approximately 200-250 gm. Same type of food (#5Purina Certified Rat Ration, and available ad libitum for all. A full-thickness excisional wound area of wound 5 cm^2 in diameter (2x2.5 cm) was excised from the dorsal aspect of all rats. The same researcher performed all surgical procedures for all rats. After surgery, the same ultrasonic therapy protocol was done for all rats in each group.

A. Comparison of mean values within each group:

Repeated measures ANOVA test revealed that; there was a significant sequential reduction in wound surface area and linear dimensions through all treatment phases in all three groups as p value < 0.05 for all. The table (1) and figure (5) shown that: there was highly significant difference between; mean of W.S.A after 3 days and mean of W.S.A before treatment, mean of W.S.A after 7 days and mean of W.S.A after 3 days, mean of W.S.A after 14 days and mean of W.S.A after 7 days in all groups .as P value (0.0001) < (0.05) for all these means. Also table (2) and figure (6) shown that; there was highly significant difference between; mean of longest dimension after 3 days and mean of longest dimension before treatment, mean of longest dimension after 7 days and mean of longest dimension after 3 days, mean of longest dimension after 14 days and mean of longest dimension after 7 days in all groups as P value (0.0001) < (0.05) for all these means. Similarly, table (3) and figure (6) shown that there was highly significant difference between; mean of widest dimension after 3 days and mean of widest dimension before treatment, mean of widest dimension after 7 days and mean of widest dimension after 3 days, mean of widest dimension after 14 days and mean of widest dimension after 7 days in all groups as P value (0.0001) < (0.05) for all these means.

Table 1: Comparison of mean of wound surface area (WSA) between every 2 sequent measuring phases within each group.

	Wound Surface Area (M±SD)			
Group	Day (0)	3rd day	7th day	14th day
C	5.0±00	3.48±0.28	1.99±0.56	0.5±0.4
P value	(0.0001)**	(0.0001)**	(0.0001)**	
Group	Day (0)	3rd day	7th day	14th day
B	5.0±00	2.96±0.69	1.82±0.16	0.28±0.16
P value	(0.0001)**	(0.0001)**	(0.0001)**	
Group	Day (0)	3rd day	7th day	14th day
A	5.0±00	2.84±1.0	0.76±0.5	0.02 ±.04
P value	(0.0001)**	(0.0001)**	(0.0001)**	

Fig. 5: Comparison of mean values of wound surface area among measuring phases in different groups.

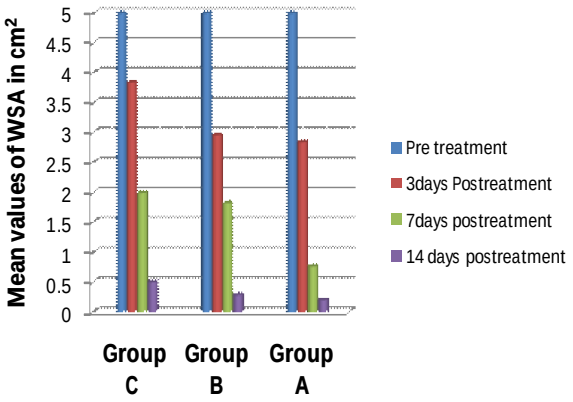


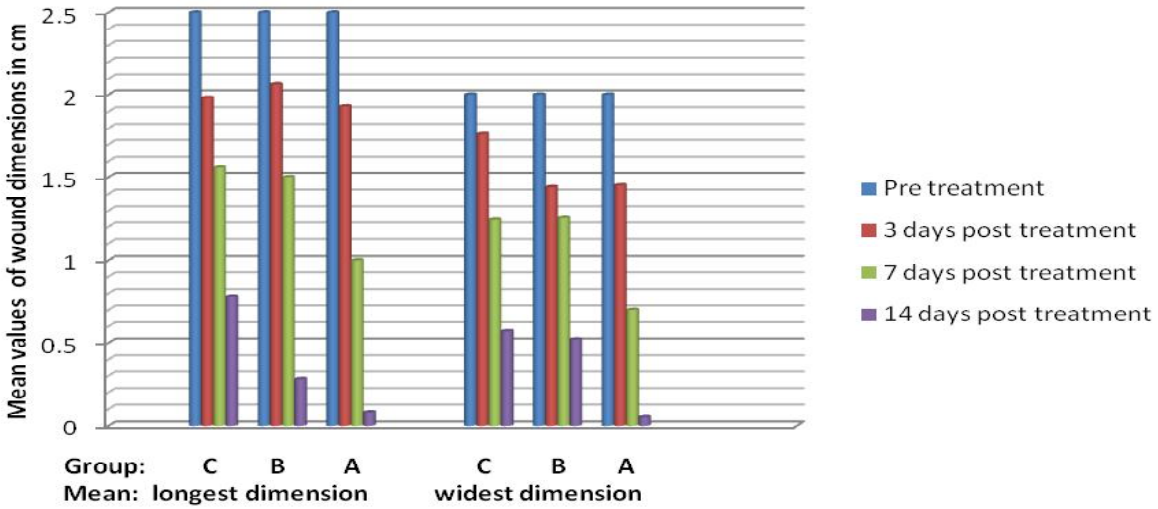
Table 2: Comparison of mean of longest dimension between every 2 sequent measuring phases within each group.

	Longest dimension (M±SD)			
Group	Day (0)	3rd day	7th day	14th day
C	2.5±00	1.98±0.10	1.56±0.33	0.78±0.22
P value	(0.0001)**	(0.0001)**	(0.0001)**	
Group	Day (0)	3rd day	7th day	14th day
B	2.5±00	2.060±.083	1.5±0.32	0.28±0.16
P value	(0.0001)**	(0.0001)**	(0.0001)**	
Group	Day (0)	3rd day	7th day	14th day
A	2.5±00	1.93±0.23	1.0±0.43	0.08±0.16
P value	(0.0001)**	(0.0001)**	(0.0001)**	

Table3. Comparison of mean of widest dimension between every 2 sequent measuring phases within each group.

	Widest dimension (M±SD)			
Group	Day (0)	3rd day	7th day	14th day
C	2±00	1.76±0.83	1.24±0.14	0.57±0.3.
P value	(0.0001)**	(0.0001)**	(0.0001)**	
Group	Day (0)	3rd day	7th day	14th day
B	2.0±00	1.44±0.31	1.25±0.2	0.52±0.29
P value	(0.0001)**	(0.0001)**	(0.0001)**	
Group	Day (0)	3rd day	7th day	14th day
A	2±00	1.45±.53	0.7±0.27	0.05±0.1
P value	(0.0001)**	(0.0001)**	(0.0001)**	

Fig. 6: Comparison of mean values of wound dimensions among measuring phases in different groups.



B. Comparison of mean values between groups

1. Wound surface area (WSA) results:

After using One way ANOVA test, the results shown in table (4) and figure (7), revealed that; there was no significant (reduction) difference between; mean of W.S.A in group C and mean of W.S.A in experimental group B, 3 days and 7 days after treatment as *P* >(0.05) value for all. While there was significant (reduction) difference between; mean of W.S.A in group C and mean of W.S.A in experimental group A, 3 days and 7days after treatment as *P* value (0.019) < (0.05).and *P* value (0.0001) < (0.05) respectively. There was significant (reduction) difference between mean of W.S.A in group C and mean of W.S.A in group B, 14 days after treatment as *P* value (0.02) < (0.05). Also there was high significant (reduction) difference between mean of W.S.A. in group C and mean of W.S.A group A 14 days after treatment as *P* value (0.0001) < (0.05).There was no significant (reduction) difference between; mean of W.S.A in group B and mean of W.S.A group A, 3days after treatment as *P* value (0.65) > (0.05).While There was high significant (reduction) difference between mean of W.S.A in group B and mean of W.S.A in group A, 7days and 14 days after treatment as *P* value (0.0001) < (0.05) and *P* value (0.008) < (0.05) respectively.

2. Wound dimensions results:

One way ANOVA test results for widest wound dimensions as shown in table (5) and figure (8) revealed that there were significant (reduction) differences between mean of widest dimension in group C and mean of Widest dimension in group B, and in group A, 3days after treatment as *P* value; (0.016) and (0.027) respectively. While there was no significant (reduction) difference between mean of Widest dimension in group C and mean of Widest dimension in group B, 7 days after treatment as *P* value (0.93) > (0.05). But there was high significant (reduction) difference between mean of Widest dimension in group C, and mean of Widest dimension in group A, 7 days after treatment as *P* value (0.0001) < (0.05). Also table (5) and figure (8) shown there was no significant (reduction) difference between mean of Widest dimension in group C and mean of widest dimension in group B, 14 days after treatment as *P* value (0.56) > (0.05).While there was high significant (reduction) difference between mean of widest dimension in group C and mean of widest dimension in group A, 14 days after treatment as *P* value (0.0001) < (0.05). Table 6 and figure (9) shown one way ANOVA results for longest dimensions and revealed that; there were no significant (reduction) differences between mean of longest dimension in group C and mean of longest dimension in group B and in group A, 3days after treatment as *P* value; (0.158) and (0.40) respectively. Also there was no significant (reduction) difference between mean of longest dimension in group C and mean of longest dimension in B, 7 days after treatment as *P* value (0.62) > (0.05).While There was high significant (reduction) difference between mean of longest dimension in group C and mean of longest dimension in group A, 7 days after treat-

ment as *P* value (0.0001) < (0.05). Also, there were high significant (reduction) differences between mean of longest dimension in group C and mean of longest dimension in group B, and in group A, 14 days after treatment as *P* value; (0.0001) for all these means.

Table 4: Comparison of means of wound surface area (WSA) between every 2 groups.

Wound Surface Area (M±SD)				
Group	pre	3 days post	7 days post	14 days post
C	5.0±00	3.48±0.28	1.99±0.56	0.5±0.4
Pvalue		-0.055	-3.303	(0.02)*
B	5.0±00	2.96±0.69	1.82±0.16	0.28±0.16
C	5.0±00	3.48±0.28	1.99±0.56	0.5±0.4
Pvalue		(0.019)*	(0.0001)**	(0.0001)**
A	5.0±00	2.84±1.0	0.76±0.5	0.02 ±.04
B	5.0±00	2.96±0.69	1.82±0.16	0.28±0.16
Pvalue		-0.65	(0.0001)**	(0.008)**
A.	5.0±00	2.84±1.0	0.76±0.5	0.02 ±.04

Table 5: Comparison of means of widest dimensions of wound every 2 groups.

Widest dimension (M±SD)				
Group	pre	3 days post	7 days post	14 days post
C	2±00	1.76±0.83	1.24±0.14	0.57±0.3.
P value		(0.016)*	-0.93	-0.56
B	2.0±00	1.44±0.31	1.25±0.2	0.52±0.29
C	2±00	1.76±0.83	1.24±0.14	0.57±0.3
P value		(0.027)*	(0.0001)**	(0.0001)**
A	2±00	1.45±.53	0.7±0.27	0.05±0.1
B.	2.0±00	1.44±0.31	1.25±0.2	0.52±0.29
P value		-0.83	(0.0001)**	(0.0001)**
A	2±00	1.45±.53	0.7±0.27	0.05±0.1

Fig. 7: Comparison of mean values of wound surface areas among different groups at each measuring phases.

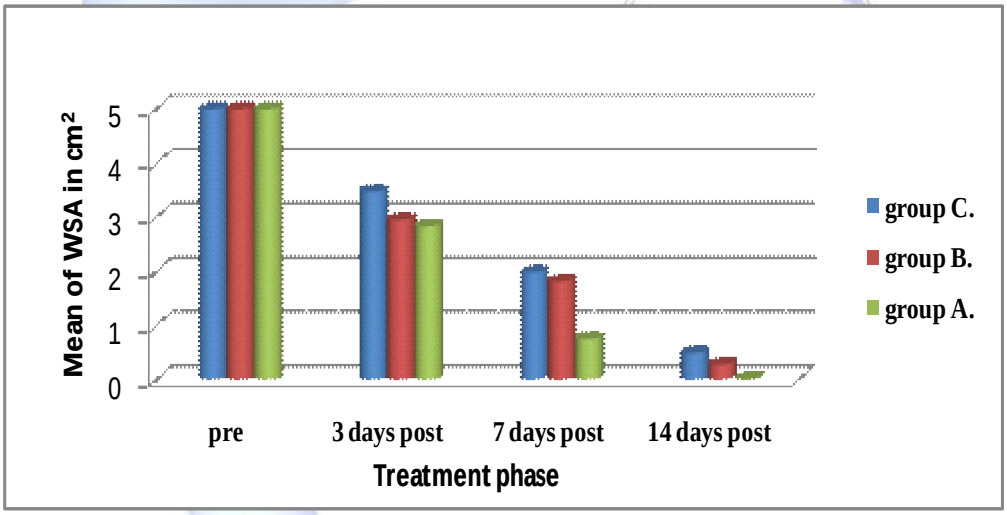


Fig. 8: Comparison of mean values of widest dimensions of wound among different groups at each measuring phase.

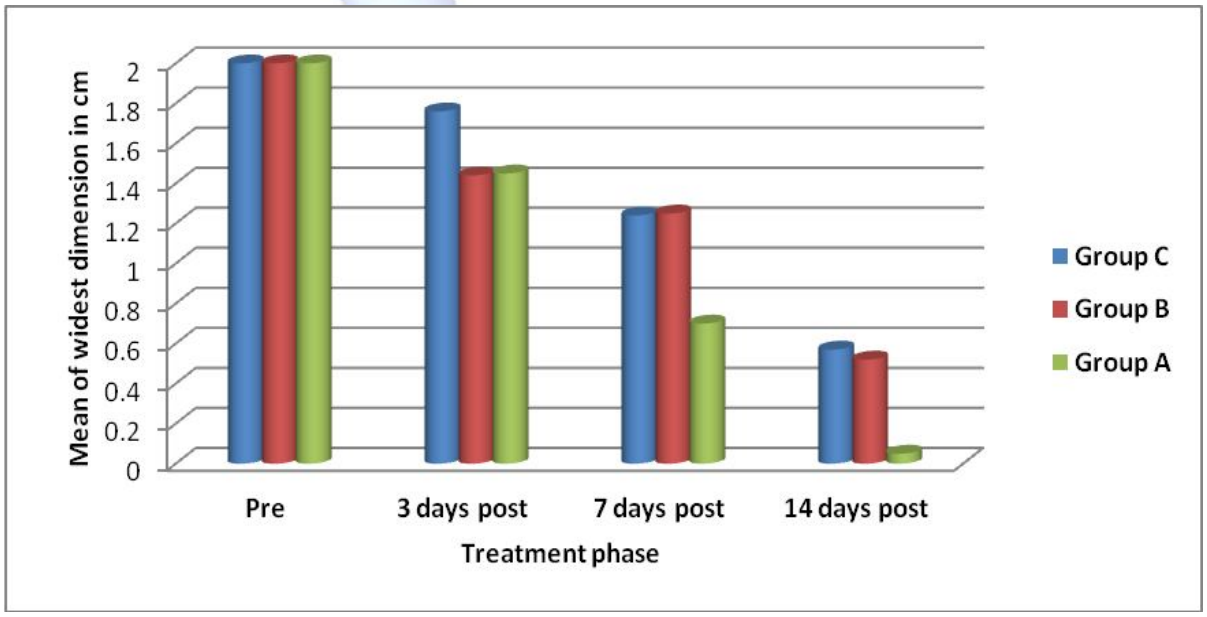
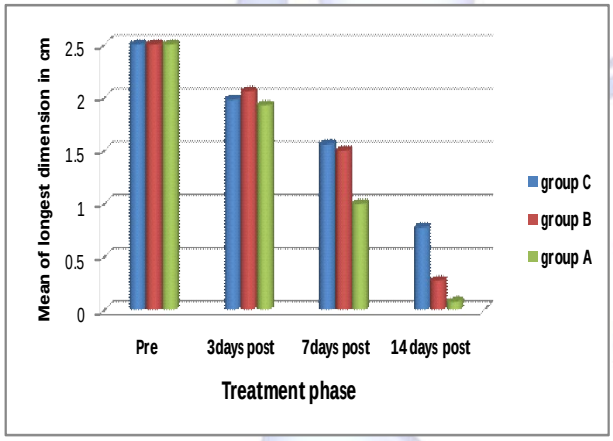


Table 6: Comparison of means of longest dimensions between every 2 groups.

Longest dimension (M±SD)				
Group	**pre**	**3 days post**	**7 days post**	**14 days post**
C	2±00	1.98±0.10	1.56±0.33	0.78±0.22
P value		-0.158	-0.62	(0.0001)**
B	2.5±00	2.060±.083	1.5±0.32	0.28±0.16
C	2.5±00	1.98±0.10	1.56±0.33	0.78±0.22
P value		-0.4	(0.0001)**	(0.0001)**
A	2.5±00	1.93±0.23	1.0±0.43	0.08±0.16
B.	2.5±00	2.060±.083	1.5±0.32	0.28±0.16
P value		(0.028)*	(0.0001)***	(0.0001)***
A	2.5±00	1.93±0.23	1.0±0.43	0.08±0.16

Fig. 9: Comparison of mean values of longest dimensions of wound among different groups at each measuring phase.



DISCUSSION

Selection of ultrasound application parameters is based on the desired effect and the location and density of the tissue to be treated. These decisions are best made by the physician and the therapist experienced in performing therapeutic ultrasound [11].The motivation of looking for alternative ultrasound parameters was due to the fact that application of high-frequency US in clinical medicine is limited due to tissue heating. Thus, using low-frequency US with less tissue heating, thereby acting as a "slow release" mechanism, may become the standard care in treating slow-to-heal lesions, skin ulcers and nonunion fractures. In addition it may be able to facilitate protein secretion and enzymatic reactions [16]. High power, high frequency ultrasound is defined as ultrasound of 0.5-10 MHz and up to1500W/cm2 while low power, low frequency ultrasound is defined as an ultrasound of 20- 120 kHz and 0.05-1.0 W/cm2). Low frequency/low intensity US is mainly reflected in the skin or wound surface. Only a small portion of the energy transmitted by the probe reaches deeper tissue layers and the major effect is mechanical effect, which is the opposite for high frequency US that combine mostly thermal with mechanical effects.[17]

A range of biological effects can be induced by ultrasound, depending on the exposure levels used. At low levels, beneficial, reversible cellular effects may be produced, whereas at high intensities instantaneous cell death is sought. The ''low power'' group includes physiotherapy, fracture repair, sonophoresis, sonoporation and gene therapy. Therapeutic effect through the intensity spectrum is obtained by both thermal and non-thermal interaction mechanisms. At low intensities, acoustic streaming is likely to be significant, but at higher levels, heating and acoustic cavitation will predominate. There is some evidence in the literature that while high intensities of ultrasound can damage bone or delay healing low intensities can enhance repair rates and reduce healing times.[18]

Callam et al used 0.5W/cm^2 (pulsed wave, frequency =1MHz) in the treatment of ulcers of various etiologies. After 12 weeks (a total of 12 procedures performed once a week) the ulcer area was reduced by 91% and healing was obtained in 30% of the cases [19]. Some promising results were obtained by Peschen et al. He treated leg ulcers with ultrasound of a power density of 0.1W/cm2 (pulsed wave, frequency=30kHz). The procedures were performed three times a week for 12 weeks (a total of 36 procedures), leading to a reduction of the ulcer area by 55% (in the placebo group the reduction was only by 16.5%)[20]. Dyson et al presents results which point to high efficiency leg ulcer treatment with ultrasound of a power density of 1W/cm^2 (pulsed wave, frequency=3MHz) in nine patients. After 4 weeks of treatment (a total of 12 procedures) the surface area of the treated ulcers was reduced by 34% as opposed to 9% in the placebo group.It seems though that these authors have based their conclusion on too small a number of subjects. Good results were obtained by Roche and West (1W/cm2, 3MHz).[21]

Eriksson et al, on the other hand, obtained un-satisfactory results from the ultrasound treatment of ulcers. Eriksson used ultrasound of a power density of 1W/cm^2 (continuous wave.

frequency=1MHz) for 8 weeks (16 procedures) [22]. Lundenberg et al used ultrasound of a power density of 0.5W/cm2 (pulsed wave, frequency=1MHz) for 12 weeks (24 procedures) [(23)]. In both cases a comparison of ulcer area did not show statistical differences between the placebo groups and ultrasound groups [22,23]. In our study, ultrasound treatment has proved to be an effective modality supporting the treatment of full thickness wound in rats. Importantly, we have demonstrated that ultra- sound of a power density of 0.5W/cm^2 (group A.) produces greater changes in the healing process in form of decreased WSA and wound dimensions than; 1W/ cm^2(group B.) and shame control group do at last two phases of treatment; 7days and 14 days post treatment. A comparison of area change with the change of linear dimensions indicates that wound area healed uniformly in all groups, ultra- sound of a power density of 0.5W/cm^2 (group A.) produces greater reduction in wound dimensions than 1W/ cm2 (group B.) and shame control group.. Although ultra- sound of a power density of 0.5W/cm^2 produce greater change in healing process than ultra- sound of a power density of 1W/cm^2, ultra- sound of a power density of 1W/cm2 produces greater changes in the healing process in comparison to control shame group as there was significant reduction of W.S.A, linear dimension was observed in group B. when compared with W.S.A , linear dimension in control group respectively in last phase of treatment (14 days post treatment).also our study showed that there were significant sequential reduction of; W.S.A and linear dimensions of wound throughout all phases of treatment in each groups.So the non-thermal effects of US, which are achieved at intensities of <0.3-1W/cm2, are gaining interest. At these levels US produces two effects, cavitation and streaming. Cavitation is the formation of gas bubbles and streaming is a unidirectional, steady mechanical force. These effects cause changes in cell membrane permeability and thus the diffusion of cellular metabolites[24]. Many laboratory-based studies have been undertaken to understand the effects of ultrasound on wound healing. To date its effects include cellular recruitment, collagen synthesis, increased collagen tensile strength, angiogenesis, wound contraction, fibroblast and macrophage stimulation, fibrinolysis, and reduction of the inflammatory phase and promotion of the proliferative phase of healing [25]. Direct effects of ultrasound have been described, such as cavitation and thermal effect. Researchers believe that the acoustic stream flow, cavitation and the associated micro stream flow are the mechanisms responsible for the non-destructive changes in the structure and function of the cellular membrane which result in greater ion permeability of the membrane.[26]

Ultrastructural changes of the protoplasm, increased intracellular calcium concentration along with stimulation of protein synthesis [27]. Release of angiogenetic factors from the macrophages (tumor necrosis factor, fibroblast growth factor) change in cellular mobility and changes in phagocyte activity (leukocytes)[28]. It has not yet been conclusively explained whether cavitation occurs in the frequency range of several MHz and several W/cm^2. Ter Haar and Daniels have observed cavitation in vivo for the therapeutic ranges (continuous wave, 0.75MHz, 0.68W/cm2). In The frequency range of 400–1000kHz, cavitation occurs at low ultrasound power densities (even 0.3–0.5W/ cm2). At higher frequencies (e.g. 20 000kHz) cavitation can only be induced with very high power densities. The experimentally determined threshold of acoustic pressure at which cavitation may occur is 0.1MPa.Some studies, where the measured acoustic pressure was 0.178MPa for a power density of 0.5W/cm2and frequency of 0.75MHz, and 0.171MPa for 3MHz, confirm that in vivo cavitation can be caused by ultrasound in the therapeutic range[29]. In another experiment, the synthesis of collagen was inhibited when a human fibroblast culture was subjected to a positive static pressure of 0.2MPa.

This indicates that cavitation was damped, as increased static pressure reduces the negative phase of the acoustic pressure at which it occurs. Despite evidence that cavitation is the main mechanism underlying the impact of ultrasound; other non- thermal mechanisms must also be taken into account (e.g. mechanical stress) as possible causes of the biological and biochemical effects. Researchers believe that the thermal mechanism of ultrasound is not involved in

regeneration. [31]

CONCLUSION

ultrasound rated at 0.5W/cm^2 as well as ultrasound rated at 1W/cm2 provide better results than control (shame) group in treatment of full thickness wound in rats, in addition the application of ultrasound rated at 0.5W/cm^2 is more beneficial than of ultrasound rated at 1W/cm2 treatment of full thickness wound in rats. So we can concluded that: The application of power densities of 0.5W/cm^2 has proved more effective in supporting healing of full thickness wound than the power density of 1W/cm2.

Conflicts of interest: None

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