Dose dependent effect of shock wave therapy on full thickness wound healing: An experimental study

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Dose dependent effect of shock wave therapy on full thickness wound healing: An experimental study

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ABSTRACT

Objective of the study: To examine the dose-effect relationship of extracorporeal shock wave therapy (ESWT) on wound healing in rats.
Study design: Randomized controlled trial design.
Animals: 45 adult male, albino rats were included in this study.
Interventions: A surgical wound with approximately six cm2 area was made on upper back of all rats after anesthesia, then rats were randomly assigned into three equal groups; group (A) (n=15, 600 shocks/session/3sessions, energy density/shock at 0.1 mJ/mm2), group (B) (n=15, 600 shocks/session/6 sessions, energy density/shock at 0.05 mJ/mm2) and group (C) (n=15, sham Shockwave group as a control group).
Outcome measures: Wound surface areas and epithelialization rates were measured at 3rd and 6th sessions by the tracing method using a digital camera.
Results: There was a significant decrease in the surface area of the wound as well as a significant increase in the epithelialization rate in three groups (p value < 0.05). Three sessions after ESWT application, group (A) showed a more significant decrease in WSA as well as a more significant increase in epithelialization rate, as compared to groups (B) and (C), whereas after three additional sessions applied in group (B), there was no significant difference between groups (A) and (B) in the main outcomes (p >0.05).
Conclusion: Shockwave dose modulation may have an effect on the end result of wound healing.

Key words: *Rats, excisional Wound, extracorporeal shock wave therapy*

Introduction

Healing of a wound is a complex process that involves different cells and events within the injured tissue. Reformation of a new vascularity is the most critical process during wound healing [1], which occurs via angiogenesis [2, 3] and new blood vessel formation [4, 5]. The incomplete healing process leads to wound infection and deterioration of the underlying tissue, which typically increase the morbidity [6].

The main wound management goal is perfect wound closure. In acute wounds, standard management includes the preparation of the wound bed, wound de-

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bridement, and surgical wound closure by skin grafts or flaps. These therapies are labor-intensive, time-consuming and expensive cost-effective. It is thus very important to seek for new efficient treatment that is less time-, labor-, and cost-consuming. Several therapies have been developed and used to treat different types of wounds, such as negative pressure therapy, ultrasonic therapy, hyperbaric oxygen therapy and Shockwave therapy [7,8].

The extracorporeal shock wave is considered as a longitudinal acoustic wave propagated in tissues, which experience a sudden change from ambient pressure to its maximum pressure. Initially, shockwave was used to break down urinary stones. Then it was used as a treatment for many orthopedic disorders such as tennis elbow, plantar fasciitis [9], long bone nonunion fractures and aseptic bone necrosis in humans [10]. Unlike the fragmentation of kidney stones, the main therapeutic objective of Shockwave therapy is not to destroy the tissue, but to stimulate tissue regeneration [11, 12]. Recently, ESWT was proven to be effective in the treatment of various wounds, including burn wounds, bedsores, vascular and diabetic ulcers [13-16].

Although the mechanism of action is still being studied, it is already known that the cavitation phenomenon of Shockwave may enhance tissue perfusion and angiogenesis, which lead to wound healing [17]. The therapeutic parameters of Shockwave used for the wound are energy density, ranging from 0.05mJ/mm^2 to 0.20mJ/mm^2, and pulse frequency ranging from 3 to 5Hz [8]. According to the energy level, ESWT can be classified into shock waves with low energy density up to 0.08 mJ/mm^2, shock waves with moderate energy density ranging from 0.09 to 0.28 mJ/mm^2 and shock waves with high energy, up to 0.6 mJ/mm^2 [18,19].

There has been no fixed protocol of treatment for shock wave therapy in wound healing because several shock wave doses have been used in different studies and most of them have a positive significant effect on healing. Saggini et al. treated different types of ulcers by using low-intensity Shockwave with energy flux density: 0.037 mJ/mm^2, and they found that there was significant improvement in healing of ulcers in the Shockwave group in comparison to wound care group [20]. Moretti et al., treated patients with diabetic ulcers by a low intensity shock wave as energy flux density was 0.03 mJ/mm^2, and there was a significantly higher healing rate in the study group compared with the control group [21]. Furthermore, Ottoman et al., applied Shockwave with medium intensity for healing donor sites after skin graft using energy density: 0.1 mJ/mm^2 and they found that the healing time was significantly faster in the ESWT comparable to time in the control group [22]. Finally, Arnó et al, found that shock wave therapy improves wound perfusion at energy density 0.15 mJ/mm^2 [23]. Given the diversity of energy densities used in past studies, our aim in this study was to examine the dose-related effect of shock wave therapy on wound healing in rats by modulating the energy density (intensity) and the treatment times.

Methods

Experimental animals:

Forty-five adult male rats, and albino type were obtained from the animal house of the Faculty of Medicine, Umm Al Qura University. At the beginning of the study, their ages were approximately 4 months, and their weight ranged from 0.2 to 0.25 kg. Each rat was individually housed in a stainless steel cage and the environment of rats was adjusted at 23-25°C and fifty percent humidity with twelve hours artificial light cycle on. Food in the form of a pellet diet (#5322 Purina Certified Rat Ration) and tap water as a source of water were continuously provided throughout the entire period of the experiment.

Excisional wound model:

The upper back area in all forty-five rats was shaved by electric clipper and disinfected using 70% concentrated alcohol. Then, all rats were anesthetized by diethyl ether inhalation. After anesthesia, the area for wound in the upper dorsal shaved skin was defined by (2x3 cm) rectangular seal, then a full-thickness excisional wound was performed and approximately 6 cm^2 of skin area was excised from all rats. The surgical procedures for all rats were done by the same researcher.

Experiment design:

A study with randomized controlled trial design was used, as after wound surgery the rats were randomly divided into three equal groups; A, B and C. (15 animals in each group) and the treatment was started within two hours after surgical procedures for each group.

Interventions

A) Treatment

All wounds were cleaned with alcohol, then sterile sonic gel filled the wound cavity in all rats. To prevent any cross-contamination of the device, a sterile plastic film was applied to all wounds and surrounding tissues. An extracorporeal shock wave device (DUOLITH® SD1, T-Top, Storz Medical Company, Tägerwilen, Switzerland) applicator (15 mm^2) with sonic gel was applied over plastic film and then moved over the wound cavity and wound margins in all groups; In the experimental group (A), Fifteen rats were received six sessions of shock wave therapy; the first 3 sessions were done with a shock wave device in the on mode and medium intensity Shockwave therapy was performed with following parameters; 600 shocks, 4 shocks per second, the energy density per shock was 0.1 mJ/mm^2 and for 150 Sec. per session, while in the last 3 sessions the device was in the off mode and for 150 Sec. per session. In the experimental group (B), fifteen rats received 6 sessions with low intensity Shockwave therapy with following parameters: 600 shocks, 4 shocks per second, the energy density per shock was 0.05 mJ/mm^2 and for 150 sec. per session, while in the control group (C) (sham Shockwave therapy) fifteen rats received Shockwave in the off mode for 150 sec. per session to control for the effects of handling and moving the shock wave applicator over the wound as well as the presence of sonic gel. In all groups, two sessions per week were done. After each session, gel and plastic film were removed and the wound was dried.

B) Measurements

1-Assessment of wound surface area:

Wound surface area (WSA) was measured using the tracing method, by placing a sterilized transparent film over each wound area, then tracing the wound shape on the film with a fine tipped marker, and placing the traced film over metric graph paper, so that the square millimeters numbers on the graph paper could be counted and converted to square centimeters. To guarantee the measurement reliability, the tracing process was repeated three times for every wound area. The mean of the three measures was calculated and considered as the wound surface area (WSA) [24]. This assessment was done at the 1^{st} , 3^{rd} and 6^{th} sessions.

2- Epithelialization rate:

The rate of healing was given by the equation (original area –unhealed area at day ^{X}) /original area x100, in which the original area is the wound surface area at day (0), while the unhealed area at day ^{X} is the traced, measured area at any day after treatment.

3- Digital camera:

The wound was photographed in the 1^{st} , 3^{rd} and 6^{th} sessions for all rats in all groups.

Statistical Procedure

The results were collected and analyzed using the SPSS program v (16), as means and standard deviations were calculated in addition to using repeated measures ANOVA as well as a paired t-test to compare mean values within a group, and one-way ANOVA was used to compare mean values between groups. The differences were considered statistically significant when the p value < 0.05.

Results

All animals were adult albino male rats approximately 4 months of age that weighed approximately 200-250 gm and were fed the same food (#5322 Purina Certified Rat Ration). One researcher did all surgical procedures for all rats so that they were homogeneous in terms of the manipulations.

Wound Surface Area (WSA) results

The results displayed in table 1 and in figure 1 show a highly significant sequential reduction in wound surface within all groups (p value < 0.0001) for all measures, as data were analyzed by repeated measures ANOVA. Table 1 also shows the results of the LSD test that revealed highly significant differences between; the WSA mean at 1^{st} session and WSA mean at 3^{rd} session, and WSA means at 6^{th} session and WSA mean at the 3^{rd} session in all groups as P < (0.0001) for all measures. In the 1^{st} session, the results in table 1 and figure 2 show that the means of WSA in all groups, approximately around 6 cm^2 and analysis of data by one-way ANOVA, have no statistically significant differences between groups as (p value = 0.85). In addition, the Post Hoc test revealed that there were no significant differences between the mean of W.S.A in group (A) and in group (B), the mean of W.S.A in group (A) and in group (C), and the mean of W.S.A in group (B) and in group (C) at 1^{st} session (p value (0.59), (0.70), (0.87), respectively).

Table 1. Comparison of wound surface area (WSA) means at (1st, 3rd and 6th sessions) within group and between groups.

	Group A	Group B	Group C	P value between	F value	Post Hoc test
1st Session	6.16±0.38	6.09±0.20	6.11±0.24	0.85	0.16	(0.59)AB (0.7)AC (0.87)BC
3rd Session	1.82±0.44	2.22±0.48	2.68±0.31	0.0001	10.63	(0.042)AB < (0.0001)AC (0.020)BC
6th Session	0.07±0.04	0.04±0.02	0.57±0.36	0.0001	19.70	(0.75)AB < (0.0001)AC < (0.0001)BC
P value within	< (0.0001)	< (0.0001)	< (0.0001)			
LSD test	< (0.0001)1st,3rd < (0.0001)1st,6th < (0.0001)3rd,6th for all groups					

(P value) AB: Group A versus Group B, (P value) AC: Group A versus Group C, (P value) BC : Group B versus Group C , (P value) 1st, 3rd : 1st session versus 3rd session, (P value) 1st, 6th :1st session versus 6th session, (P value) 3rd, 6th :3rd session versus 6th session.

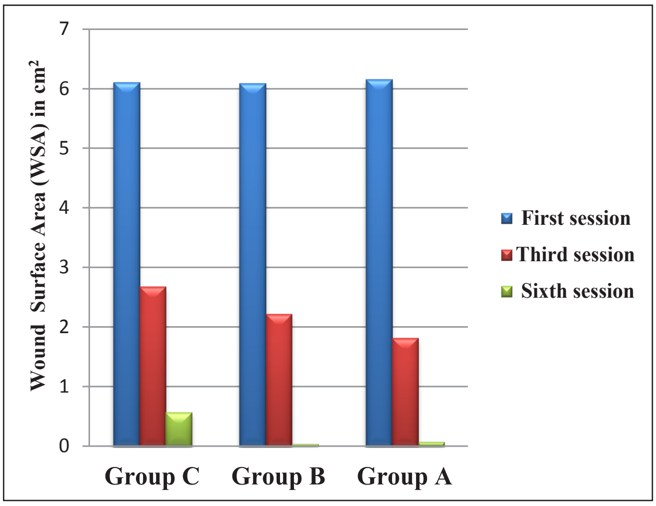

Figure 1. Comparison of wound surface area (WSA) means within each group.

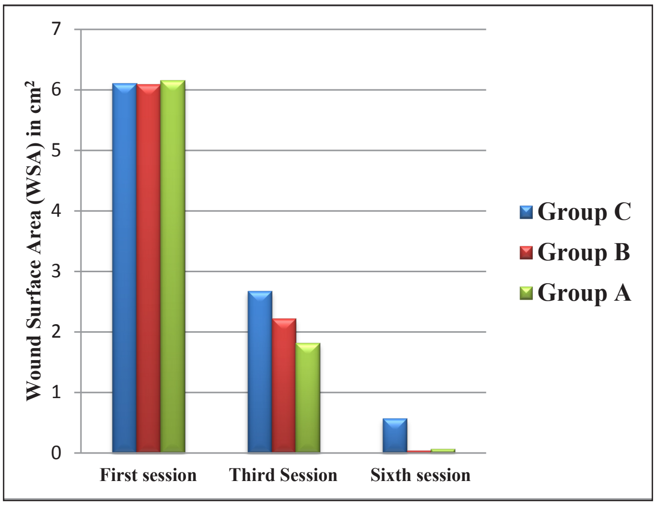

Figure 2. Comparison of wound surface area (WSA) means between groups at each phase of assessment.

At 3rd session (table 1 and figure 2), the mean of the WSA in group (A) was 1.82±0.44 cm², while it was 2.22±0.48 cm² in group (B) and it was 2.68±0.31 cm² in group (C); the analysis of these data revealed that there was a highly significant difference between the groups (p value < 0.0001). Detailed analysis of the data revealed that; there were significant differences between; the mean of W.S.A in group (A) and that in group (B), and between the mean of W.S.A in group (B) and that in group (C) at 3rd session as p values (0.042), (0.020) respectively, also there was a high significant difference between the mean of W.S.A in group (B) and that in group (C) at 3rd session as p value < (0.0001).

At 6th session (table 1 and figure 2) the mean of the WSA in group (A) was 0.07±0.04 cm², while it was 0.04±0.02 cm² in group (B) and it was 0.57±0.36 cm² in group (C). Analysis of data revealed a highly significant difference between the groups as (p value < 0. 0001). Post Hoc tests revealed that there were highly significant differences between the mean of W.S.A in group (A) and in group (C) and the mean of W.S.A in group (B) in group (C) after the 6th session (p value < 0.0001 for all measures), while there was no significant difference between men of W.S.A in group (A) that in the group (B) (p value = 0.75). Figure 3 shows examples of captured images by a digital camera of the wound surface area (WSA) providing comparative images for wounds within each group and between groups at 1st , 3rd and 6th sessions.

Rate of epithelialization results

The results displayed in a table 2 and in figure 4 revealed that at 3rd session the epithelialization rate mean in the group (A) was 70.25 ±7. 84 %, while it was 63.5 ±7. 73 % in the group (B) and it was 55.6 ±5. 25 % in the group (C). Analysis of data by one-way ANOVA revealed that there was a highly significant difference be-

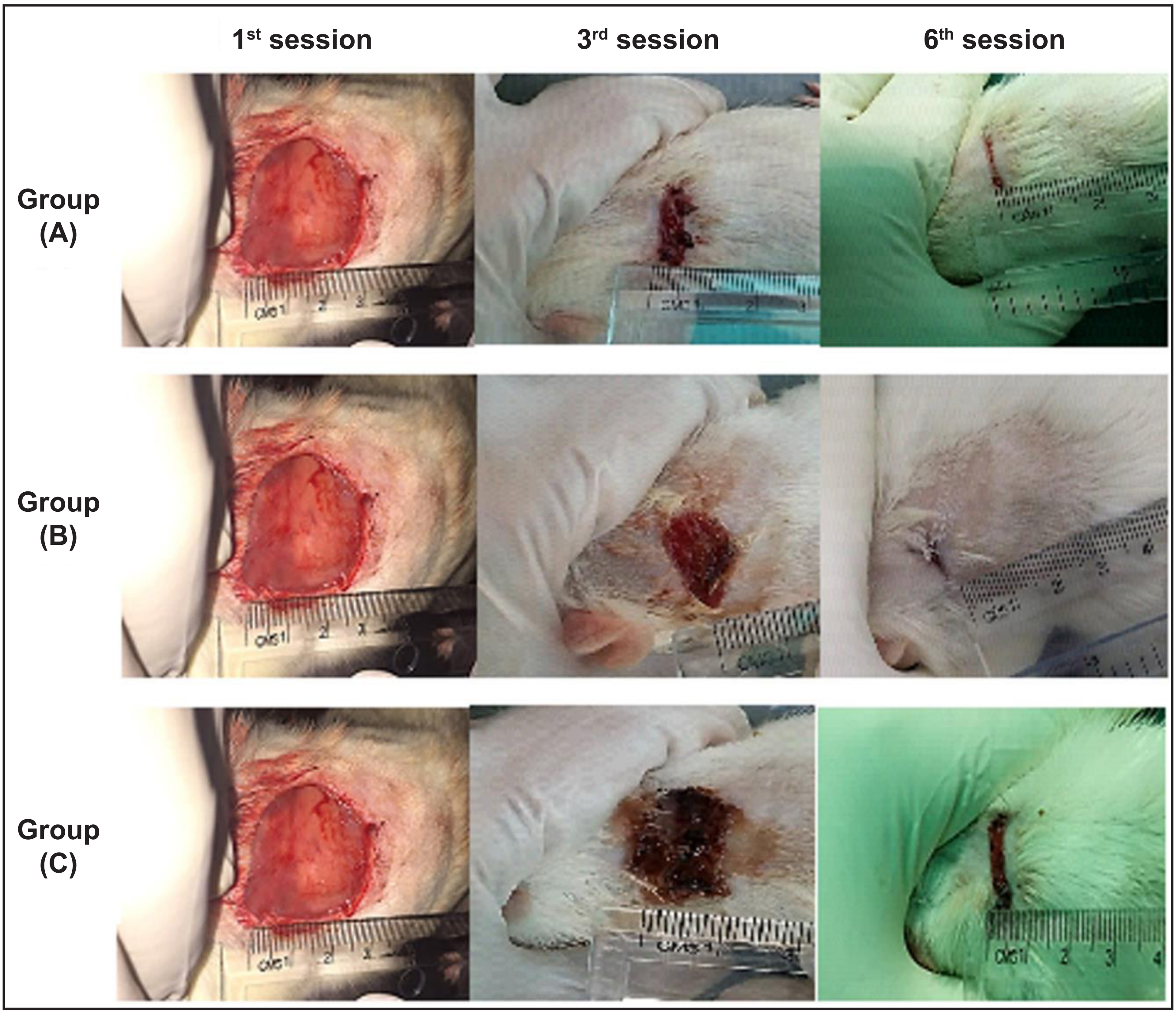

Figure 3. Examples of captured images by digital camera of the wound surface area (WSA) at (1st), (3rd), and (6th) sessions within three groups.

Table 2. Comparison of epithelialization rate means within group and between groups.

	Group C	Group A	Group B	P value between	F value	Post Hoc test
3rd Session	55.6 ±5.25	70.24 ±7.84	63.5 ±7.7	<0.0001	10.24	$(0.04)^{AB}$, $< (0.0001)^{AC}$ $(0.024)^{BC}$
6th Session	90.7±5.9	98.8±0.67	99.3 ±0.34	<0.0001	19.9	$(0.75)^{AB}$, $< (0.0001)^{AC}$ $< (0.0001)^{BC}$
P value within	$< (0.0001)^{3rd,6th}$ for all groups					

$(P\ value)^{AB}$: Group A versus Group B, $(P\ value)^{AC}$: Group A versus Group C, $(P\ value)^{BC}$: Group B versus Group C.

tween the groups ($p < 0.0001$). Data analyzed by Post Hoc test revealed that there were significant differences between the epithelialization rate mean in group (A) and that in group (B), the epithelialization rate mean in group (A) and in group (C), and the epithelialization rate mean in groups(B) and in group (C) at 3rd session (p value =0.04, < 0.0001,0.024), respectively.

At 6th session, table 2 and figure 4 show that the epithelialization rate mean in group (A) was 98.8±0.67 %, while it was 99.3 ±0.34 % in group (B) and it was 90.7±5.9 % in group C; there was a highly significant difference between the groups ($p < 0.0001$). Post Hoc test revealed that there were significant differences between the epithelialization rate mean in group (A) and

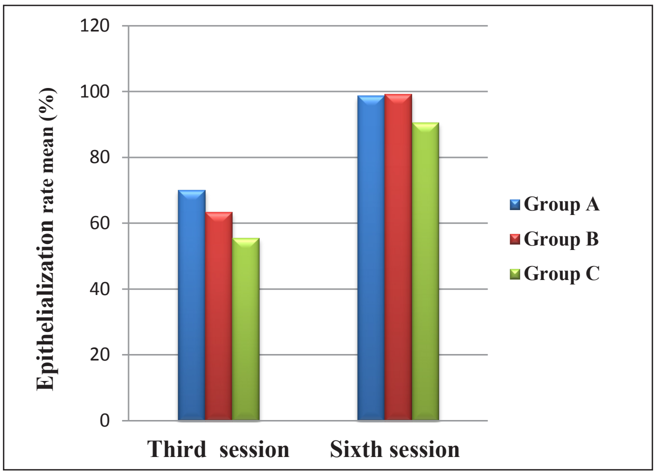

Figure 4. Comparison of epithelialization rate mean between groups at each phase of assessment.

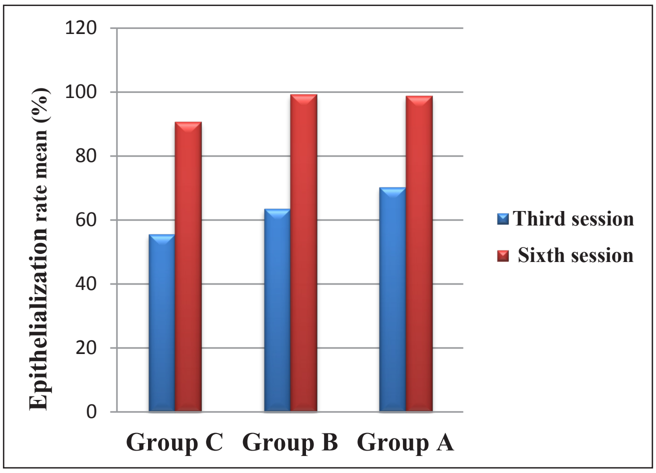

Figure 5. Comparison of epithelialization rate mean within each group.

that in group (C) and the epithelialization rate mean in group (B) that in group (C) at the 6th session ($p < 0.0001$) for all measures, while there was no significant difference between epithelialization rate mean in group (A) and in group (B) (p value = 0.75).

Table 2 and figure 5 show that the comparison of epithelialization rate means between two phases of assessment (3rd and 6th sessions) within each group using paired t-test revealed a highly significant difference between the 3rd and 6th sessions within three groups (p value < 0.0001 for all measures).

Discussion

In our study an excisional wound model was employed for the assessment of the dose-effect relationship of shock wave therapy on wound healing in rats. The results of this study provided evidence that adjustment of both the duration and intensity of treatment affect the wound healing process and also determined which dose has a better healing effect. Notably, the comparison between groups at 3rd session revealed that the healing process in both groups (A) and (B) in the form of shrinking of wound surface and healing rate was significantly better than in group C. This can be explained by the shock wave therapy effect on the healing process and this result is consistent with previous studies. In fact, recent studies confirmed that ESWT may be useful and effective in wound healing as it stimulates several endogenous growth factors in experimental animals [25-28]. It facilitates the recruitment of endothelial progenitor cells [29], and angiogenesis induction [30, 31].

ESWT improves tissue perfusion through the increasing nitric oxide, which is considered as a strong vasodilatation mediator. Shockwave facilitates nitric oxide production through increasing expression of NO synthase. Shockwave strongly facilitates the induction of vascular endothelial growth factor (VEGF), which is the most potent vasculogenic and pro-angiogenic substance [32]. The increased expression of receptors of vascular endothelial growth is also noted in targeted tissues aer shockwave therapy [33].

In the wound tissue treated with Shockwave, suppression of pro-inflammatory cytokines and chi-machines, and expression facilitation of several genes of wound healing were found [26, 34]. Several studies have confirmed that Shockwave has an anti-inflammatory effect as early local inflammatory responses were found in wound tissues of ESWT animal group [25, 35]. At the local wound tissue, ESWT facilitates cell proliferation, stimulates the extracellular matrix metabolism, and decreases apoptosis [29, 36].

The results of the 3rd session revealed that group (A) had better wound healing than group (B), as there was a highly significant reduction in the mean WSA as well as a highly significant increase in the epithelialization rate mean in group (A) when compared with the mean WSA and epithelialization rate mean in group (B), respectively. These results may be attributed to shockwave energy density per shock, as while both groups received equal duration of treatment (3 sessions), group (A) received energy density (0.1mmJ/cm^2) per shock, which is twice the dose received by group (B) (0.05mmJ/cm^2).

At 6th session, the comparison between groups revealed that the healing process with respect to the shrinking of wound surface and the healing rate was significantly better in both groups (A) and (B) than in group C. This can be explained by a continuous shock wave therapy effect until the last session in group (B), while in group (A) a non-therapeutic spontaneous healing at the last 3 sessions occurred due to the supporting and propulsion effect of therapeutic healing at the first 3 sessions with duplicated dose.

Interestingly, at 6^{th} session , there was no significant difference between group (A) and group (B) in the mean WSA as well as in the epithelialization rate mean. At that time point, the same total amount of energy was received by wounds in both groups, as in group (A) the shock wave therapy was in off mode during the last 3 sessions, while wounds in group (B) received energy density untill the last (6^{th}) session but in half dose compared to group (A). Thus, the number of treatment sessions is another factor to consider in wound healing. Our results confirmed that the shockwave therapy effect on the healing of wound depends on the times of treatment as well as the energy flux density (intensity) of treatment. They also revealed that shock wave energy flux density at 0.1mmJ/cm^2 is better than 0.05 mmJ/cm^2 in accelerating wound healing and it was confirmed as the intensity of choice in the several previous studies discussed below.

A study on the effect of ESWT on the healing of 2^{nd} degree burns in rats demonstrated that the ESWT group (n=15) received Shockwave at an energy flux density of 0.11 mJ/mm^2 , while the control group (n=15) did not receive any treatment. Wound closure and epithelialization rate in the ESWT group were increased compared to control group ($p < 0.05$) [37].

A study compared Shockwave therapy and hyperbaric oxygen therapy in the treatment of diabetic foot ulcers. In the ESWT group, an energy density of 0.1 mJ/mm^2 was applied as one session every two weeks to the diabetic foot ulcer. Ninety-minute application of HBOT was performed using a multiple-choice chamber at 2.5 atmospheric pressure. Thirty-one percent (31%) of the ESWT group reached complete ulcer healing compared with 22% of the HBOT group, which was a statistically significant difference; in addition, a more than 50% improvement of wound surface was observed in 89% of the shock wave group compared with 72% of the HBOT group [38].

In a clinical study, forty-four patients with acute 2^{nd} degree burns were randomly distributed into two groups of the same size (22 patients each), that received burn wound debridement and Shockwave therapy (experimental therapy) or no therapy (control group). After debridement, one session of ESWT with energy density level at 0.1 mJ/mm^2 was applied to the study group and the results revealed that one session of shock wave applied to the 2^{nd} degree burn significantly improved healing [39].

In an animal study, diabetic rats with wound model were used to investigate the effect of ESWT on wound healing and collagen content in tissues of the wound. 100 impulses of shock wave at an energy density level: 0.11 mJ/mm^2 were applied to the wound in ESWT groups. No Shockwave was administered in non-diabetic and diabetic groups. On 7 and 14 days post-wounding, rats were sacrificed. After ESWT administration, the fibroblasts and the new collagen fibers were significantly increased at the wound site and transforming growth factor expression was also up-regulated [40].

Conclusion

Shockwave dose modulation may affect the end result of wound healing. Therefore, both of energy density (intensity) and treatment times are important factors that should be considered when treating with shock wave therapy.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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