Comparison of the Effect of High Voltage Pulsed Galvanic Stimulation versus Ultrasonic on the Acceleration of Burn Wound Healing

Emad T. Ahmed*, Mohamed M. Abdel-khalek Khalaf*
*Lecturers of Physical therapy Department of Surgery, Faculty of Physical Therapy, Cairo University

ABSTRACT

The purpose of this study aimed to compare the effect of high voltage pulsed galvanic current (HVPG) versus ultrasonic (US) on the burn wound healing. Thirty females adult New Zealand rabbits (4-6 months of age) weighing between 2 and 2.5 kilograms were used in this study. The rabbits were randomly assigned into three groups; Group I: (ten rabbits) received HVPG current continuously for one hour a day by negative polarity for the first three days and positive polarity for the second three days. The same dosage and application time were used with negative polarity on the seventh day. Group II: :(ten rabbits) received pulsed US treatment applied by moving applicator technique, for five minutes daily for seven days. Group III: (ten rabbits) represented the control group which received standard medical wound care. ALL three groups received traditional physical therapy program. Assessment was done by measuring wound surface area (WSA) and measurement of Hydroxyproline content. The results of this study showed that there was a significant reduction in WSA in the HVPG and US group when compared with WSA in the control group ($p<0.05$).Also, There was a significant increase in hydroxyproline content in HVPG and US group when compared with hydroxyproline in the control group ($p<0.05$). There was a significant reduction in WSA in the HVPG group when compared with WSA in the US group ($p<0.05$). There was a significant increase in hydroxyproline content in HVPG group when compared with hydroxyproline in the US group ($p<0.05$). The clinical implications of this study are that HVPG stimulation and US may increase the rate of burn wound healing. The addition of HVPG stimulation and US treatment to wound care program may promote more rapid healing, thus decrease treatment time, cost and decrease the length of institutional stay of patients.

INTRODUCTION

Humans have sought various means to facilitate wound healing for all of recorded history, with the goals of speeding the healing process, preventing infection, maximizing wound strength, minimizing scaring and preventing disability[6].

The basic principle on which electrotherapy is based remain simple through which a wide range of application can be derived, each of which has its place in patients care. At its most fundamental levels the application of an external energy to the tissues can results in the activation, stimulation or enhancement of physiological activity in particular tissues, depending on the mode of energy that has been applied[16].

Numerous physical therapy approaches to wound healing are described, including ultrasound (US), and High Voltage Pulsed Galvanic Stimulation (HVPG), but many questions have been raised regarding the best treatment tools in the acceleration of burn wound healing.

Ultrasound may work at several levels in the early stages of healing; US may decrease edema[11], trigger mast-cell degranulation[9,10] and increase blood flow, secondarily increasing the delivery of oxygen and

macrophages to the area[1]. In the later stages of healing US may stimulate collagen deposition[12] and remodeling[9,10].

Most of our knowledge of the effects of ultrasound on living tissue has been gained through in vitro studies animal models, and much of this research has focused in particular upon skin wound and ulcers[15].

It has been suggested that, ultrasound interacts with one or more components of inflammation and earlier resolution of inflammation, accelerated fibrinolysis, stimulation of macrophage-derived fibroblast mitogenic factors, heightened fibroblast recruitment, accelerated angiogenesis, increased matrix synthesis, dense collagen fibrils and increased tissue tensile strength have all been demonstrated in vitro. Such findings form the basis for the use of ultrasound to promote and accelerate tissue healing and repair[15].

Electrical stimulation has long been recommended as an adjunct treatment for wound healing. Some investigators[3,5,8,17], described the use of HVPG stimulation for acceleration of wound healing. These modalities were believed to enhance wound healing by stimulating growth of granulation tissue and by producing bactericidal effect[4,14].

Recently, the effects of HVPG stimulation and US were reported individually in terms of acceleration of wound healing and the development of good quality scar.

This study was aimed to compare the effect of US and HVPG stimulation on the burn wound healing. Ultrasonic has a short application time as opposed to the HVPG stimulation in clinics. A comparison was made in order to investigate differences in the application times and the effect in burn wound healing measured by changes in hydroxyproline amounts, and wound surface area (WSA) between US and HVPG stimulation.

MATERIAL AND METHODS

Animals

Thirty females adult (4-6 months of age) New Zealand rabbits weighing between 2 and 2.5 kilograms (Kg) were purchased from a local animal house and housed, one per standard cage, the rabbits were randomly assigned to numbers that was printed on the ears, with indelible ink. Animals remained in their cages, except during treatment set-up, where food and water were available. Temperature (22^0C) and the ratio of daylight hour (12 hours) to non daylight hours (12 hours) were kept constant.

Induction of Burn

Each animal was anesthetized by placing the animal into closed cage in association with a piece of cotton wetted with ether. The animals were strictly tied via four cotton threads to a wooden plate. The hair at the upper part of the left hind limb was removed by hair removal cream (Veet), manufactured by EVA, Cosmetic, Egypt and the skin were cleaned by a piece of cotton wetted with alcohol. The area of the skin prepared as above equal three square centimeters Cm^2, while the area intended to be burned equal two Cm^2, however, this will ease the procedure of burning as well as measurement. The burns were induced by a rectangular metal seal with a two Cm^2 contact surface area that was heated on a flame burner to 45^0 C, and handled by a surgical forceps, then pressed immediately against the prepared skin segment for 20 seconds to produced partial thickness burns[2].

All rabbits were randomly divided into three groups

Group I: HVPG stimulation treatment (An Intelect model 500-S) HVPG stimulation treatment device was used, carbon rubberized electrodes were placed on pads moistened with saline solution. Since the active electrode was placed on the wound, the passive one was placed distally. The HVPG stimulation of (100 µsec, 150V, and 100Hz) was applied continuously for one hour a day by negative polarity for the first three days and positive polarity for the second three day. The same dosage and application time were used with negative polarity on the seventh day; ten rabbits were used in this group.

Group II: Ultrasound treatment (A sonopulse 463, Enraf-Nonius) device was used with a probe of eight mm, in diameter and sterile sonsgel (Enraf- Nonius) . A pulsed US of (2msec on, 8msec off, and 0.1W/Cm^2 intensity) was applied by moving applicator technique, for five minutes daily for seven days. Ten rabbits were used in this group.

Group III: Control group, this group received standard wound care consisting of debridement by qualified personal and application of non adherent gauze pads. Both experimental groups received standard wound care in addition to US wound or HVPG stimulation. All the wounds were cleaned with sterile saline solution before and after treatment.

Evaluation Procedures

Measurements of Wound Surface Area (WSA)

Wound surface area was calculated by placing a piece of sterilized transparency film over the burn wound and tracing the wound perimeter on the film with a fine tipped transparency marker. A separate transparency was used for each wound. The tracing was placed over metric graph paper and the number of one mm^2 within the tracing inside counted (only full on millimeters squares inside the perimeter) were counted and the area was counted to squared centimeter). The measurement was taken after 24 hours post burn induction (Pre-treatment), and post one week. Each measurement was taken three times for the same burn wound and the mean value of the three measurements was calculated for more reliability.

Measurement of Hydroxyproline Content

The wound surface area was gently wiped off with gauze before sampling, but no attempt was made to separate eschars from the wounds. Full thickness skin biopsy specimens (mean wet weight, 0.092 gm) that extended to the subcutaneous fat layer were obtained with a 6 mm diameter punch from the center of the wounds. The biopsy specimens were put on ice and then stored at-70^0C, until analysis was done for hydroxyproline (day one –day seven). Uninjured skin was also harvested with a 6 mm punched before burning to detect normal hydroxyproline level. The skin biopsy specimens were hydrolyzed in 6NHCL for 18 hours at 110^0C, and analyzed for total hydroxyproline content colorimetrically. The hydroxyproline contents are expressed as milligrams per square centimeter[7].

Data analysis

For each group WSA and hydroxproline content of the treatment groups wounds and the control wounds were compared with paired t -test. Comparison of each group with other group was made by independent t -test. The level of significant was set at 0.05 for all statistical tests.

RESULTS

The results of this study are presented under the following headings:

I-Results of HVPG group

The mean value and standard deviation of WSA (Cm^2) in this group before application of the treatment was (2.01±0.02 Cm^2), while the mean values of WSA after application of HVPG measured after one weak were (0.92±0.15Cm^2). There was a significant decrease in the WSA measured after one week post stimulation of HVPG compared to initial measurement before application of treatment, with ($P<0.01$), as shown in table (1), and fig (1).

The mean value of hydroxyproline content in this group before application of treatment was (11.13 ±0.84mg/Cm^2), the mean value of hydroxyproline content in this group after 24 hours post-burn induction and before application of the treatment was (3.7 ±0.34mg/Cm^2), while the mean value of hydroxyproline content after application of HVPG measures one week was (5.72 ±0.36mg/Cm^2), as shown in table (2), and fig (2).

There were a significant decrease in hydroxyprline content measured after 24 hours post burn induction compared to initial measurement (before burn induction) ($P<0.05$). On the other hand there was a significant increase in hydroxyproline content measured after 7 days post application of HVPG compared to 24 hours post burn induction ($P<0.001$), as shown in table (2), and fig (2).

II-Results of Ultrasonic group

The mean value of WSA (Cm^2) in this group before application of the treatment was (2.05±0.15 Cm^2), while the mean value of WSA after application of US measured after one weak was (0.12±0.18 Cm^2). There was a significant decrease in the WSA measured after one week post stimulation of US compared to initial measurement before application of treatment, with ($P<0.01$), as shown in table (1), and fig (1).

The mean value of hydroxyproline content in this group before application of treatment was (11.12 ±0.88mg/Cm^2), the mean value of hydroxyprline content in this group after 24 hours post-burn induction and before application of the treatment was (3.74 ±0.32mg/Cm^2), while the mean value of hydroxyproline content after application of US measures one week was (8.56 ±0.57mg/Cm^2), as shown in table (3), and fig (3).

There was a significant decrease in hydroxyproline content measured after 24 hours post burn induction compared to initial measurement (before burn induction) ($P<0.05$). On the other hand there was a significant increase in hydroxyproline content measured after 7 days post application of US compared to 24 hours post burn induction ($P<0.001$), as shown in table (3), and fig (3).

III-Results of Control group

The mean value of WSA (Cm^2) in this group before treatment was (2.01±0.03 Cm^2), while the mean value of WSA after one weak were (1.51 ±0.06 Cm^2). There was a significant decrease in the WSA measured after one week when compared to initial measurement before treatment, with ($P<0.01$), as shown in table (1), and fig (1).

The mean value of hydroxyproline content in this group before treatment was (11.1 ±0.79mg/Cm^2), the mean value of hydroxyprline content in this group after 24 hours post-burn induction and before the treatment was (3.75±0.29mg/Cm^2), while the mean value of hydroxyproline content after

measures one week were (4.58 ±0.33mg/Cm^2), as shown in table (4), and fig (4).

There was a significant decrease in hydroxyproline content measured after 24 hours post burn induction compared to initial measurement (before burn induction) (P<0.05). On the other hand there was a significant increase in hydroxyproline content measured after 7 days post when compared to 24 hours post burn induction (P<0.001), as shown in table (4), and fig (4)

Table (1): The statistical analysis of mean value of WSA for Groups (I, II, and III).

	WSA(Cm^2)					
	G I (HVPG)		GII (US)		G III (Control)	
	Before treatment	Post one week	Before treatment	Post One week	Before treatment	Post One week
X	2.01	0.12	2.05	0.92	2.01	1.51
SD	0.02	0.18	0.15	0.15	0.03	0.06
MD	1.09		1.93		0.5	
T-value	24.6		48		20	
P-Value	0.001		0.001		0.001	
Significant	Sig.		Sig.		Sig.	

X=Mean SD=Standard deviation MD=Mean differences P -value=Probability level.

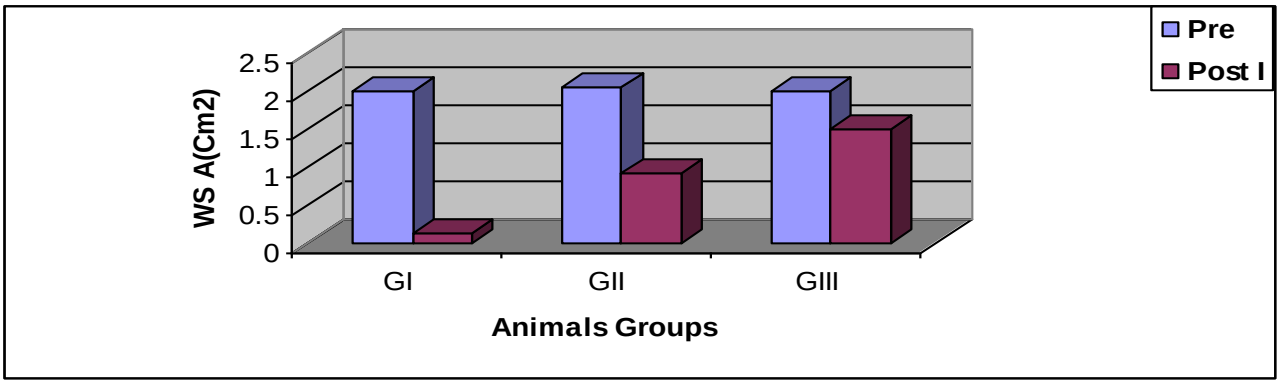


Fig. (1): Shows the mean value of WSA for Groups (I, II, and III).

Table (2): The statistical analysis of mean value of hydroxyproline for Group I (HVPG).

	Level of Hydrosyproline for Group I(HVPG) (mg/cm^2)					
	Before treatment	After 24 hours	Before treatment	Post One week	After 24 hours	Post One week
X	11.13	3.7	11.13	8.56	3.7	8.56
SD	0.84	0.34	0.84	0.36	0.34	0.36
MD	7.43		5.41		-2.02	
T-value	27		16.77		11.46	
P-Value	0.001		0.001		0.001	
Significant	Sig.		Sig.		Sig.	

X=Mean SD= Standard deviation MD=Mean differences P-value=Probability level.

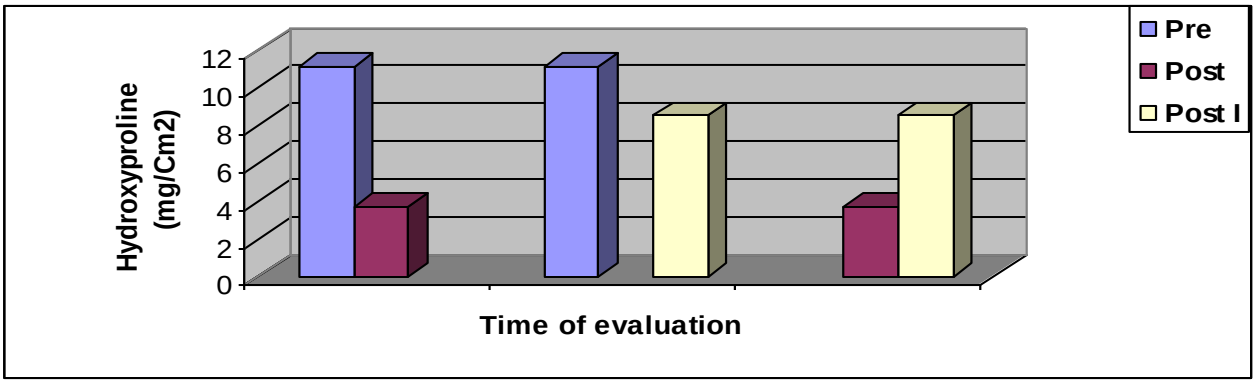


Fig. (2): Shows the mean value of hydroxylproline group I (HVPG).

Table (3): The statistical analysis of mean value of hydroxyproline for Group II (US).

	Level of Hydrosyproline for Group II(US) (mg/cm^2)					
	Before treatment	After 24 hours	Before treatment	Post One week	After 24 hours	Post One week
X	11.12	3.74	11.12	5.72	3.74	5.72
SD	0.88	0.32	0.88	0.36	0.32	0.36
MD	7.383		2.56		-4.82	
T-value	24.34		6.96		27.85	
P-Value	0.001		0.001		0.001	
Significant	Sig.		Sig.		Sig.	

X=Mean SD= Standard deviation MD=Mean differences P-value=Probability level.

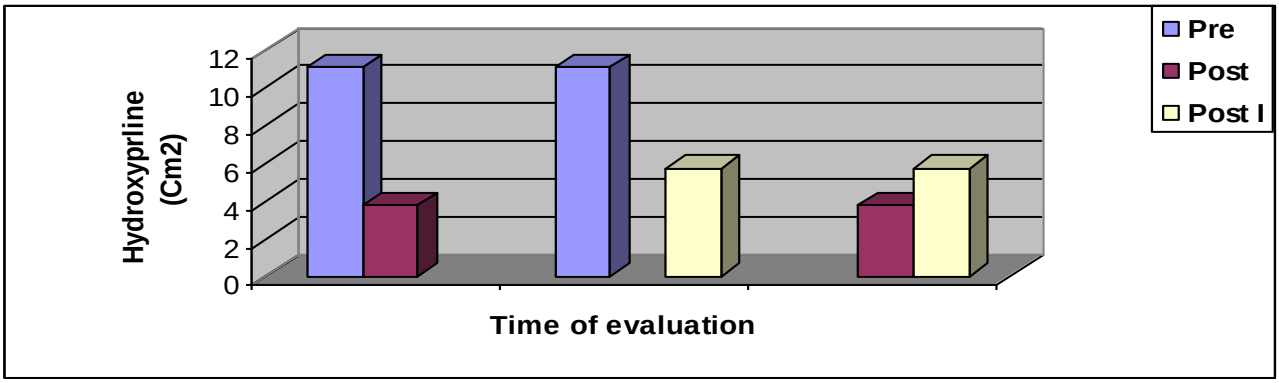


Fig. (3): Shows the mean value of hydroxylproline group II(US).

Table (4): The statistical analysis of mean value of hydroxyproline for Group III (Control).

	Level of Hydrosyproline for Group III(Control) (mg/cm^2)					
	Before treatment	After 24 hours	Before treatment	Post One week	After 24 hours	Post One week
X	11.1	3.75	11.1	4.58	3.75	4.58
SD	0.79	0.29	0.79	0.33	0.29	0.33
MD	7.35		6.52		-0.83	
T-value	31.48		23.62		6.04	
P-Value	0.001		0.001		0.001	
Significant	Sig.		Sig.		Sig.	

X=Mean SD= Standard deviation MD=Mean differences P-value=Probability level.

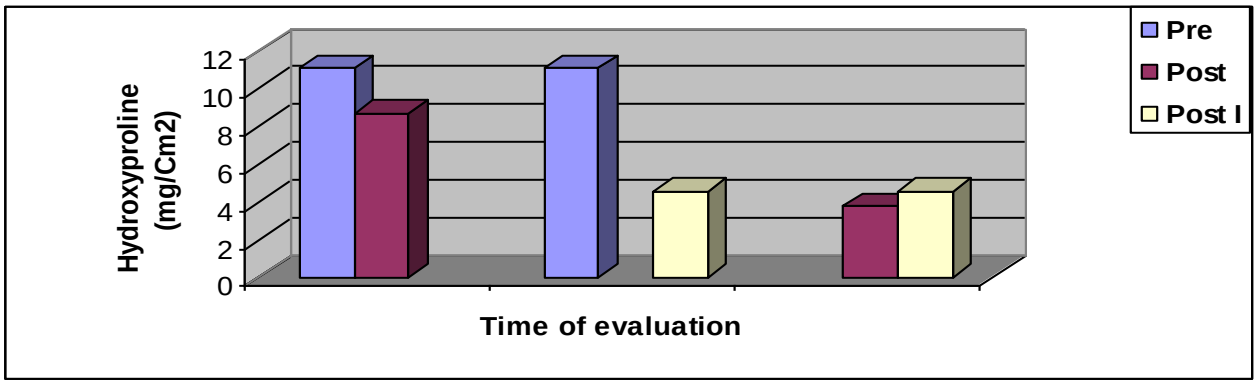


Fig. (4): Shows the mean value of hydroxylproline group III (control).

Comparison and analysis of the mean values of WSA and hydroxyprline content after three different groups of rabbits (after one week)

This part of comparison for three different groups (control, US, and HVPG groups) was analyzed and suggested the following results.

There was a significant reduction in WSA in the US group when compared with WSA in the control group, as in table (5), and fig (5).

On the other hand there was a significant increase in hydroxyproline content in US group when compared with hyderoxyproline in the control group, as in table (6), and fig (6).

There was a significant reduction in WSA in the HVPG group when compared with WSA in the control group, as in table (5), and fig (5).

On the other hand there was a significant increase in hydroxyproline content in HVPG group compared with hyderoxyproline in the control group, as in table (6) and fig (6).

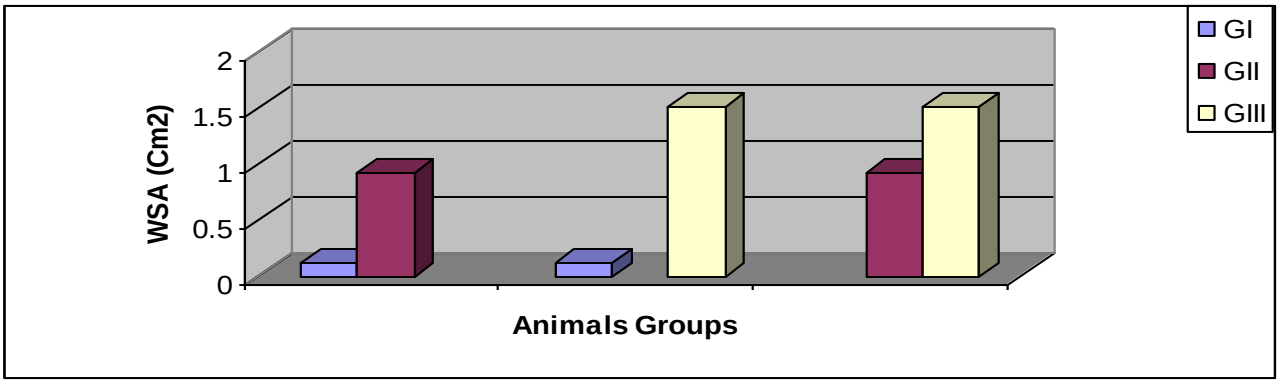
There was a significant reduction in WSA in the US group when compared with WSA in the HVPG group, as in table (5), and fig (5).

On the other hand there was a significant increase in hydroxyproline content in US group when compared with hyderoxyproline in the HVPC group, as in table (6), and fig (6).

Table (5): The statistical analysis of mean value of WSA after one week of treatment application between groups of the study.

	WSA(Cm^2)					
	GI (HVPG)	GII (US)	GI (HVPG)	GIII (Control)	GII (US)	GIII (Control)
X	0.12	0.92	0.12	1.51	0.92	1.51
SD	0.18	0.15	0.18	0.06	0.15	0.06
MD	0.8		-0.58		-139	
T-value	10.59		10.97		22.56	
P-Value	0.001		0.001		0.001	
Significant	Sig.		Sig.		Sig.	

X=Mean SD= Standard deviation MD=Mean differences P-value=Probability level
GI=HVPG stimulation group GII= US group GIII=Control group.



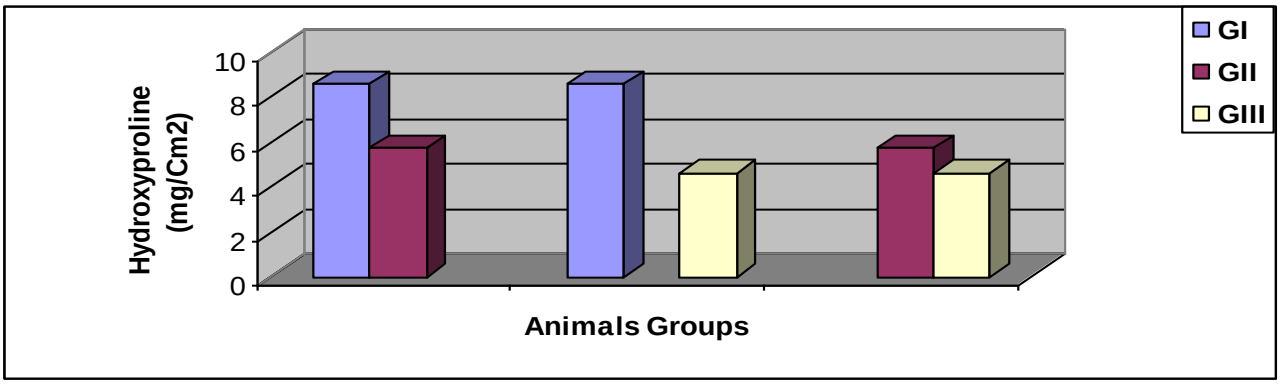
Time of evaluation after (one week)

Fig. (5): Shows the mean value WSA after one week of treatment application between groups of the study.

Table (6): The statistical analysis of mean value of hydroxyproline after one week of treatment application between groups of the study.

	Level of Hydrosyproline(mg/Cm^2)					
	GI	GII	GI	GIII	GII	GIII
X	8.56	5.72	8.56	4.58	5.72	4.58
SD	0.57	0.36	0.57	0.33	0.36	0.33
MD	-2.84		1.14		3.98	
T-value	13.18		7.34		18.88	
P-Value	0.001		0.001		0.001	
Significant	Sig.		Sig.		Sig.	

X=Mean SD= Standard deviation MD=Mean differences Pvalue=Probability level
GI=HVPG stimulation group GII= US group GIII=Control group.



Time of evaluation after (one week)

Fig. (6): Shows the mean value of hydroxyproline after one week of treatment application between groups of the study.

DISCUSSION

Recently, the beneficial effects of HVPG stimulation and US treatment were shown in wound healing **.**According to the protocol used in this study US takes five minutes in application and HVPG takes one hour in application, if both US and HVPG stimulation have the same effect on wound healing, US therapy will be the best methods in clinics.

We study whether collagen synthesis could be triggered on the seven day of wound healing by either HVPG stimulation or US. We found that HVPG stimulation was better than US on the promotion of wound healing measured either by hydroxyproline content (measure of collagen) or WSA.

Recent study have shown that the cells migrating across a wound came from a strip of intact skin approximately 0.5mm wide around the wound and substantial voltage gradient occurs in this area. The cell migration mechanisms have not been understood yet. But their movement under the influences of voltage gradient might be one possible mechanism, for example by electrophoresis or by active polarization of charged molecules in the cell membranes[7].

To explain this, there is suggestion that the recorded current in the experimental wounds triggers the wound healing. Because of this effect exogenous electrical current was used in order to accelerate wound healing **(13).**

The changes in wound healing that occurred over the study period were evaluated using measurement of WSA and hyroxyproline level. These measurements were taken by a single observer who was blinded to the treatment groups thus we control and reducing rater bias.

These measurements of WSA and hydroxyproline level were taken after one week of treatment. This direction of treatment is consistent with that used in other clinical trials of other wound care treatment. Pervious studies have demonstrated that one week of treatment is sufficient to evaluate the efficiency of wound treatment.

Conclusion

The clinical implications of this study are that HVPG stimulation and US may increase the healing rate of burn wound healing. The addition of HVPG stimulation and US treatment to wound care program may promote more rapid healing, thus decrease treatment time, treatment cost, and length of institutional stay of patients.

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الملخص العربى

مقارنة التيار الجلفاني المتردد العالي الفولتيه
مقابل الموجات فوق الصوتية علي تسريع التئام جروح الحروق

تهدف هذه الدراسة إلي مقارنة التيار الجلفاني المتردد العالي الفولتيه مقابل الموجات فوق الصوتية علي التئام جروح الحروق. وقد اجريت هذه الدراسة علي ثلاثون أرنب نيوزلاندي (4-6 شهور من العمر) يزنوا من 2 الي 2.5 كيلو جرامات. قسمت الأرانب عشوائيا الي ثلاث مجموعات ، المجموعة الأولي وعددها (10 أرانب) طبق عليهم التيار الجلفاني المتردد العالي الفولتيه لمدة ساعة واحدة بالتقاطب السلبي للثلاث أيام الأولي و بالتقاطب الأيجابي للثلاث أيام التالية و بالتقاطب السلبي بنفس الجرعة والوقت لليوم السابع . المجموعة الثانية و عددها (10 أرانب) طبق عليهم الموجات فوق الصوتية الترددية لمدة خمس دقائق يوميا لمدة سبع أيام.المجموعة الثالثة وعددها (10 أرانب) وهي المجموعة الحاكمة طبق عليهم العناية الطبية للجرح .أجرى التقييم بقياس سطح الجرح ومحتوى الهيدروكسى بروليين . أظهرت النتائج انخفاض ملحوظ في سطح الجرح في مجموعتي التيار الجلفاني المتردد العالي الفولتية و الموجات فوق الصوتية بالمقارنة بالمجموعة الحاكمة . هناك ازدياد ملحوظ في محتوى الهيدروكسى بروليين في مجموعتي التيار الجلفاني المتردد العالي الفولتية و الموجات فوق الصوتية بالمقارنة بالمجموعة الحاكمة . أظهرت النتائج أيضا انخفاض ملحوظ في سطح الجرح في مجموعة التيار الجلفاني المتردد العالي الفولتية بالمقارنة بمجموعة الموجات فوق الصوتية بينما هناك ازدياد ملحوظ في محتوى الهيدوركسى بروليين في مجموعة التيار الجلفاني المتردد العالي الفولتيه بالمقارنة بمجموعة الموجات فوق الصوتية . وقد أستنتجنا من هذه الدراسة أن التيار الجلفاني المتردد العالي الفولتية والموجات فوق الصوتية من الممكن أن تزيد من نسبة التئام جروح الحروق وأن أضافة التيار الجلفاني المتردد العالي الفولتيه و الموجات فوق الصوتية الي برنامج العناية لجروح الحروق تساعد علي سرعة شفاء الجروح و تبعا تقلل من وتكلفة العلاج وأيضا مدة بقاء المريض بالمستشفي.