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The Synergistic Action of Laser and Photosensitizer on *staphylococcus aureus* Wound Infection in Mice

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الخلاصة

صممت هذه الدراسة لتحديد تأثير الليزر الدايودي لوحده ومع المضاد الحيوي سيفوتاكسيم ومحلول اليود الكحولى كمحسس للضوء فى التئام الجروح اجريت الجروح فى الفئران بطول1سم فى اضهرها ولوثت الجروح بالمكورات العنقودية الذهبية.

تم تقسيم الفئران المجروحة الى تسع مجاميع عرضت للاشعاع الليزرى نصف دقيقة و1 و2 و3 دقائق كل ثلاثة ايام وعولجت بالسيفوتاكسايم لوحده، محلول اليود الكحولي لوحده، محلول اليود مع السيفوتاكسيم، محلول اليود مع الليزر، السيفوتاكسايم مع الليزر، مع السيفوتاكسايم مع اليود الكحولى.

الليزر مع محلول اليود الكحولي و السيفوتاكسايم يزيد سرعة التئام الجروح بشكل معنوي مقارنة مع المجموعات الاخرى. احسن النتائج معنويا سجلت عنداستخدام الليزر ومحلول اليود الكحولى مع السيفوتاكسيم. ان التعريض لليزر يقلل التركيزالمثبط الادنى للمضاد الحيوى ضد المكورات العنقودية الذهبية. احسن فترة تعريض عجلت من التئام الجروح هى نصف دقيقة كل ثلاثة ايام. يحدث تسريع التئام الجروح نتيجة لاحد أو اكثرمن الاسباب الاتية: اما قتل الليزر للمكورات العنقودية الملوثة مباشرة او التأثير على امراضيةالمكروب او تغيير مقاومة الجراثيم للمضادات الحيوية او التداخل مع حيثيات التئام الجروح مؤدية الى زيادة سرعة الالتئام.

من خلال هذة الدراسة يمكن الاستنتاج من ان الليزر قد يشكل علاجا جديدا، وولغرض اثبات هذا الاستنتاج على الانسان نحتاج الى تجريب سريرى لتحديد فعاليتة على الانسان.

Abstract

This study was designed to determine the effect of diode laser alone and in combination with cefotaxime and povidone-iodine (as photosensitize) on wound healing. Wounds (1 cm in length were induced in the backs of the mice). Wounds were infected with *S. aureus*. Wounded mice divided to nine groups and treated by laser (as one exposure for 0.5, 1, 2, 3 minutes each three days), cefotaxime alone,

povidone iodine alone, povodine iodine and cefotaxime, povodine iodine and laser, cefotaxime and laser, and laser- cefotaxime- povodine iodine combination. Laser in combination with either povidone iodine or cefotaxime significantly accelerate wound healing compared with other groups. The best significant results were obtained with the using of laser- povidone iodine- cefotaxime combination. Laser exposure also minimizes the minimum inhibitory concentration of the antibiotic against *S. aureus* when it exposed to it. The best period of exposure in vivo was found to be 0.5 min once exposure each 3 days. The acceleration of wound healing induced by laser could be attributed toone or more of the following its bactericidal effect on the contaminated microorganism, its effect on the pathogenicity of these organism, changing of resistance pattern of the contaminants, or its interference with the events participated in wound healing and subsequent increase in the rate of the healing. This study proved that laser is a good therapyfor healing injuries; therefore clinical trials are required to assay its efficacy in human being.

Introduction

Photodynamic therapy (PDT) is based on the dye- sensitized photo oxidation of biological matter in the target tissue [1]. This requires the presence of a dye (sensitizer) in the tissue to be treated. Although such sensitizers can be naturally occurring constituents of cells and tissues, in the case of (PDT), they are introduced into the organism as the first step of treatment. In the second step, the tissue-localized sensitizer is exposed to light of wavelength appropriate for absorption by the sensitizer [2].

Laser has found enormous applications in various fields of biology and medicine[3,4]. The biological effects of laser radiation have been studied for years, low-energy laser beams can be used as assimilative tool in the living system [5]. Such radiation can interact with biological substances at various molecular and atomic and macroscopical changes [3, 6, 7]. Repeated exposure to low- energy red laser beams was observed to have a stimulatory effect on healing in several types of wounds [8].

The aim of the study is an attempt to assess the therapeutic effects of laser with and without photo sensitizer (povodine iodine) and cefofaxime in the rate of healing of the experimentally induced…wound in mice.

Materials and Methods

Antibiotic susceptibility testing:

A loopful growth from all bacterial isolates was inoculated into nutrient broth and incubated at 37ºC for 18 hrs. The bacterial suspensions were diluted with a sterile ringer solution. The proportion of dilution was 1:1000. One ml of bacterial suspension was poured on to the surface of the Mueller Hinton agar plate (Oxoid,

U.K), and left for ten minutes to settle the bacteria. The excess of bacterial suspension were discarded using Pasteur pipette. The plates were kept for one hour at room temperature to dry, were used by sterile forceps which flamed after being cleaned with alcohol. The diameter of inhibition zones was measured utilizing the method of Bauer et al [10].

Determination of minimal inhibitory concentration (MIC) of antibiotic for *S. aureus*:

A doubling dilution of each antibiotic in Mueller Hinton ager plates A loopful of each *S. aureus* cultures was inoculated into tubes containing sterile nutrient broth and incubated overnight at 37ºC. Dilution of broth cultures was done up to 100- fold with nutrient broth. All the tubes were inoculated with diluted broth cultures of *S. aureus* and incubated at 37ºC for 24 hrs. The results were read to the end of visible.

Identification of *Staphylococcus aureus*:

The suspected colonies of Staphylococcus aureus identified following the conventional method [10].

The effect of laser light exposure on *S. aureus* in vivo:

Animals:

Four hundred and twenty Swiss albino mice of 25 gm weight were taken from the laboratory of the biological and pharmaceutical quality control (Baghdad). They were housed in the animal house of Tikrit collage of medicine. The animals were kept at room temperature adjusted to 25ºC; they were allowed food and water ad.libitum [11].

Laser:

The laser diode with measured output at 5mW (laser Beacon, I.N.C. Michigan, U.S.A) was used in the present study.

Photosensitizers:

Povidon – iodine (I.C.I, Britain) was used in concentration of 16µg/ml.

One day prior to infection, mice were anesthetized by ether anesthesia [12,13] the back of the mice were closely shaved with a fine-tooth electric clipper. On the day of infection, wounds were produced on the backs of reanesthesized mice [14] by making a longitudinal midline incision one cm in length and extending down to the paniculus carnosus. The wound was infected by taking a drop of Pasteur pipette by seeding containing 105 CFU of *S. aureus* [12].

Treatment design:

After induction of the wound mice were divided into nine groups as follows:

1 - First group: 80 mice were subdivided into 4 subgroups and treated as follows:

a - First subgroup: 20 mice were treated by intramuscular injection of 3 mg cefotaxime (16.67 mg / kg B.W. /day) as a single daily injection. The wound was flooded with photosensitizer (povodine iodine) and exposed to

laser radiation for 0.5 minute, once exposure each three days.

b - Second subgroup: 20 mice were treated as above, but the period of exposure to laser increased to 1 minute.

c - Third subgroup: 20 mice were treated as above, but the period of exposure to laser increased to 2 minutes.

d - Fourth subgroup: 20 mice were treated as above, but the period of exposure to laser increased to 3 minutes [15,16].

2 - Second group: 80 mice were subdivided into 4 subgroups and treated as follows:

a - First subgroup: 20 mice were treated by intramuscular injection of 3 mg cefotaxime (16.67 mg/kg B.W./day) as a single daily injection, and the wound was exposed to laser radiation for 0.5 minute, once exposure each three days.

b - Second subgroup: 20 mice were treated as shown in (a) but the period of exposure to laser increased to 1 minute.

c - Third subgroup: 20 mice were treated as shown in (a) but the period of exposure to laser increased to 2 minutes.

d - Fourth subgroup: 20 mice were treated as shown in (a) but the period of exposure to laser increased to 3 minutes.

3 - Third group: 80 mice were subdivided into 4 subgroups and treated as follows:

a - First subgroup: 20 mice, the wound was flooded with photosensitizer (Povodine iodine) and exposed to laser radiation for 0.5 minute, once exposure each three days.

b - Second group: 20 mice were treated as in (a) but the period of exposure to laser increased to 1 minute.

c - Third subgroup: 20 mice were treated as in (a) but the period of exposure to laser increased to 2 minutes.

d - Fourth subgroup: 20 mice were treated as in (a) but the period of exposure to laser increased to 3 minutes.

4 - Fourth group: 20 mice were treated by 3 mg cefotaxime (16.67 mg/kg B.W./day) as a single daily intramuscular injection, and the wound was flooded with povodine iodine.

5 - Fifth group: 20 mice were treated by cefotaxime only as 3 mg (16.67 mg / kg B.W. /day) injected intramuscular as a single daily dose.

6 - Sixth group: 20 mice were treated by flooding of the wounds by povodine iodine.

7 - Seventh group: : 80 mice were subdivided into 4 subgroups and the wound was exposed to laser radiation for 0.5, 1, 2, 3 minutes respectively as one exposure each three days.

8 - Eighth group: 20 mice in which the wound left without treatment.

9 - Ninth group: 20 mice in which the wound was uncontaminated by *S. aureus* and left without treatment [15, 16].

The treatment in all groups was continued till complete healing. The length of the wound was estimated daily. Healing rate represented the reduction in the length of the wound.

Statistical analysis:

Single sided student t- test was used to estimate the significancy among groups, and among subgroups.

Results

Sensitivity before exposure to laser:

Our study before exposure to laser showed that all isolates (4) were resistant to tetracycline, ampicilin and 1 of 4 isolates was resistant to gentamicin and cefotaxime. However 2 isolates were resistant cloxacillin and amoxicillin and all isolates were sensitive to cefalothin.

Sensitivity after exposure to laser:

After exposure to laser-povidone iodine combination, only one isolate stay resistant to tetracycline, and cefotaxime, 2 isolates stay resistant to gentamicin and cefalothin and 3 isolates stay resistant to tetyracycline and cloxacillin.

Minimum inhibitory concentration:

Our study showed that minimal inhibitory concentration of the four *S. aureus* isolates for cefotaxime was 2± 0.08μg/ml.

Effect of laser exposure alone and in combination with cefotaxime and povidine iodine on the rate of wound healing:

The rate of the healing on wound contaminated by Staphylococcus aureus and exposed to laser radiation for 0.5 minutes, once exposure each 3 days in combination with cefotaxime and povidone iodine was greater than the healing rate in the wound exposed to laser for 1, 2, and 3 minutes with the same combinations. However this variation was not significant except between the healing rate of the wound exposed to laser radiation for 0.5 minute, and that exposed to laser for 3 minutes ($p< 0.05$) (Table 1). Healing rate of the wound exposed to laser for 0.5 minute in combination cefotaxime povidone iodine was significantly ($p<0.01$) more than the healing rate of wound in mice treated by cefotaxime 0.5 minute laser exposure, povidone iodine 0.5 minute laser exposure and more significantly ($p<0.001$) than the healing rate of wound in mice treated by cefotaxime alone, povidone iodine alone, their combination, laser alone, and control untreated group(Table 10).

No significant variations were recorded among the healing rates in mice treated by cefotaxime with 0.5, 1, 2, 3 minutes of laser exposure respectively (Table2).

No significant variations were recorded between this combination and povidone iodine – laser combination. The healing rate of wounds treated by this combination was significantly more than cefotaxime alone ($p<0.01$), povidone iodine alone ($p<0.01$), a combination of cefotaxime and povidone iodine alone ($p<0.01$), laser alone ($p<0.05$) and control untreated group ($p<0.001$) (Table 10).

No significant variations were recorded among the healing rates in mice treated by cefotaxime with 0.5, 1, 2, minutes of laser exposure. However the healing rate in wound exposed 0.5 minute laser exposure in combination with povidone iodine was more ($p< 0.05$) than the healing rate of wound treatment by povidone iodine with 3 minutes laser exposure.(Table 3). However although the healing rate in this group was less ($p<0.01$) than those treated by cefotaxime - povidone iodine laser combination, but the healing rate in this group was significantly more than the healing rate of the wounds in mice treated by cefotaxime povidone iodine combination ($p<0.001$), cefotaxime alone ($p<0.001$), povidone iodine alone ($p<0.001$), laser alone ($p<0.01$) and control untreated wound ($p<0.001$) (Table 10).

The healing rate of the staphylococcus aureus contaminated wound in mice treated by cefotaxime povidone iodine combination was 0.74±0.33mm./day (Table4). This healing rate is significantly less than that of mice treated by cefotaxime povidone iodine laser combination ($p<0.001$), cefotaxime laser combination ($p<0.01$) and povidone – laser combination ($p<0.001$). However the variations in the healing rate in this group were not significant compared with the using of cefotaxime alone, povidone iodine alone, laser alone in control untreated group (Table 10).

The healing rate of the Staphylococcus aureus contaminated wound in mice treated by cefotaxime alone was 0.71±0.22mm./day (Table 5). This healing rate is significantly less than of mice treated by cefotaxime- povidone iodine- laser combination ($P < 0.001$), cefotaxime – laser ($P < 0.001$).

However, there was no significant variation among the healing rate in this group and the group treated by povidone iodine alone laser alone and control unteated group (Table 10). The healing rate of the Staphylococcus aureus contaminated wound in mice treated by povidone iodine alone was 0.61±0.19mm./day (Table 6). This healing rate is significantly less than the healing rate of wound in mice treated by cefotaxime- povidone iodine-laser combination ($P< 0.001$), povidone iodine – laser combination ($P < 0.001$). However, there was no significant variation among the healing rate of the wound in this group and that of mice treated by povidone iodine- cefotaxime combination, cefotaxime alone, laser alone and in control untreated group (Table 10).

The rate of the healing in wound contaminated by staphylococcus aureus and exposed to laser radiation for 0.5, 1, 2, 3 minute, once exposure each 3days were

0.86±0.49, 0.82±0.32, 0.74±0.32 and 0.70±0.46 mm/day. However the variations in the healing rates among subgroups were not significant. The healing rate of the wound in this group is significantly less than of mice treated by cefotaxime-povidone iodine-laser combination ($P < 0.001$), cefotaxime-laser combination ($p<0.05$), and povidone iodine laser combination ($P < 0.01$). However the healing rate of the wound in this group was not significantly differ than the healing rate in mice treated by povidone iodine-laser combination, cefotaxime alone, povidone iodine alone, and in control untreated group (Table10).

The healing rate of the Staphylococcus aureus contaminated wound in untreated mice was 0.61±0.21mm/day and the healing rate in uncontaminated untreated wound was 0.73±0.21mm/day between these two groups was not significant. However, the healing rate in both groups was only significantly less than that of mice treated by cefotaxime- povidone iodine-laser combination ($P < 0.001$), cefotaxime-laser combination ($p<0.01$), and povidone iodine laser combination ($P < 0.001$) (Table10).

Treatments	No. of animals	Rate of healing									Mean± SD*
		Days									
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th	
Cefotaxime and povidone iodine with laser exposure for 0.5 min.	20	2.36	2.96	2.12	2.30						2.50±0.23 a
Cefotaxime and povidone iodine with laser exposure for 1 min.	20	1.70	0.32	2.32	2.42	3.16					1.98±1.06 a b
Cefotaxime and povidone iodine with laser exposure for 2 min.	20	1.70	0.60	1.38	1.7	4.06	2.36				1.96±1.17 a b
Cefotaxime and povidone iodine with laser exposure for 3 min.	20	1.88	1.88	1.58	2.14	1.16	1.56	1.86	0.1		1.46±0.67 b c

* Similar letter means: Not significant

Table 1: Rate of healing of Staphylococcus aureus infected wound in mice treated by cefotaxime, povidone iodine with different periods of exposure to laser radiation.

Treatments	No. of animals	Rate of healing									Mean ± SD*
		Days									
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th	
Cefotaxime and povidone iodine with laser exposure for 0.5 min.	20	1.56	1.08	1.28	1.52	1.80					1.45±0.27
Cefotaxime and povidone iodine with laser exposure for 1 min.	20	1.30	1.40	0.26	0.84	0.78	0.94	2.12			1.9±0.58
Cefotaxime and povidone iodine with laser exposure for 2 min.	20	1.36	1.08	0.44	0.89	0.38	0.62	2.06			0.96±0.59
Cefotaxime and povidone iodine with laser exposure for 3 min.	20	1.54	0.96	0.64	0.94	0.94	1.02	1.20			1.8±0.28

*The variations among groups were not significant

Table 2: Rate of healing of Staphylococcus aureus infected wound in mice treated by cefotaxime with different periods of exposure to laser radiation.

Treatments	No. of animals	Rate of healing									mean±SD*
		Days									
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th	
povidone iodine with laser exposure for 0.5 min.	20	1.48	1.66	1.62	1.49	1.53					1.56±0.08 A
povidone iodine with laser exposure for 1 min.	20	1.12	1.32	1.26	1.29	1.42	1.22				1.27±0.10 a b
povidone iodine with laser exposure for 2 min.	20	1.22	1.21	116	1.61	1.61	1.28	1.26			129±0.16 a b
povidone iodine with laser exposure for 3 min.	20	1.42	1.32	1.26	1.18	1.05	1.01	1.12			1.19±0.14 B

* Similar letter means: Not significant

Table3: rate of healing of staphylococcus aurous infected wound in mice treated by povidone iodine with different periods of exposurete laser radiation.

Days of the treatment	Healing rate mm.																			Mean
1st. day	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	0.02	0.03	0.04	0.05	0.9	0.8	0.7	0.06	0.5	0.4	0.47
2nd.day	1.9	1.3	1.3	1.3	0.8	1.7	1.7	1.7	1.7	1.7	2.78	2.87	2.07	2.17	1.34	1.45	2.60	0.6	0.6	0.7	1.58
3rd.day	1.0	1.6	0.5	0.6	0.9	0.3	0.3	0.3	0.4	0.7	0.7	0.2	1.89	1.68	1.56	1.55	0.3	2.30	2.3	2.3	1.06
4th.day	0.5	0.5	1.0	1.0	0.9	0.8	0.3	0.3	0.2	0.6	0.4	0.5	0.4	0.7	0.8	1.0	1.3	0.5	0.7	1.6	0.75
5th.day	1	1.0	1.9	0.9	0.8	0.8	1.5	1.0	1.8	0.9	0.9	1.1	0.6	0.5	0.5	0.5	0.4	0.7	1.7	1.2	0.95
6th.day	0.5	1.5	0.3	0.6	1.7	0.8	0.4	0.7	0.2	0.4	0.6	0.8	0.7	0.7	0.7	0.7	0.6	0.3	0.2	0.8	0.74
7th.day	0.4	0.6	0.6	0.4	0.4	0.5	0.8	1.0	0.7	0.7	0.7	0.5	0.4	0.4	0.4	0.8	1.0	0.8	0.9	0.6	066
8th.day	0.7	0.5	0.4	0.7	0.7	0.5	0.4	0.6	0.6	0.8	0.8	1.0	0.9	0.7	0.6	1.1	0.5	1.1	.9	0.5	0.70
9th.day	0.8	0.7	0.7	0.7	0.9	1.5	1.4	1.5	1.6	1.1	1.0	0.7	0.9	0.6	1.0	0.2	0.8	0.4	0.6	1.7	0.97
10th.day	0.6	0.7	0.7	0.5	0.3	0.7	0.8	0.5	0.6	0.9	0.3	0.5	0.4	0.7	0.5	0.6	0.6	0.6	0.6	0.3	0.52
11th.day	0.6	0.4	0.6	0.4	0.4	0.4	0.4	0.4	0.3	0.3	0.4	0.7	0.8	1.0	0.6	0.4	0.5	0.5	0.5	0.6	0.53
12th.day	0.05	0.1	0.05	0.6	0.17	0.3	0.1	0.2	0.02	0.1	0.3	0.2	0.1	0.5	0.3	0.8	0.5	0.3	0.4	0.3	0.26
13th.day	0.85	0.6	0.45	0.1	0.53	0.4	0.6	0.8	0.88	0.1	0.5	0.8	0.7	0.2	0.6						0.54

No. of animals: 20 **Mean ±SD 0.74±0.33**

Table 4: Rate of healing of Staphylococcus aureus infected wound in mice treated by cefotaxime and povidon iodine.

Days of the treatment	Healing rate mm.																			Mean
1st. day	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.20	1.19	1.21	1.22	1.24	1.25	0.88
2nd.day	0.8	0.2	0.2	0.2	0.5	0.3	0.42	0.33	0.24	0.15	0.06	0.17	0.08	0.19	0.20	0.60	0.5	0.05	0.04	0.04	0.22
3rd.day	0.2	0.8	0.8	0.8	0.5	0.2	0.83	0.77	0.66	0.55	0.83	0.61	0.59	0.57	0.10	0.75	0.62	0.60	0.58	0.69	0.55
4th.day	0.8	0.8	1.0	1.7	1.7	1.9	1.2	1.10	1.2	0.9	0.9	0.9	0.9	1.5	1.	1.1	1.12	1.12	1.14	1.02	0.84
5th.day	0.6	0.6	0.7	0.4	0.7	0.8	0.85	0.6	1.1	0.3	0.5	0.7	0.9	1.1	0.7	0.8	1.1	1.1	0.9	1.0	0.98
6th.day	0.5	0.5	0.4	1.0	0.7	0.9	1.2	0.5	0.9	0.7	0.5	0.7	0.7	0.8	1.0	1.0	1.1	1.1	1.3	1.5	0.78
7th.day	0.4	0.4	0.6	0.4	0.5	0.5	0.9	0.9	0.9	0.9	0.8	0.9	0.7	0.6	0.4	0.2	0.7	0.7	0.7	1.4	0.67
8th.day	0.8	0.8	0.6	0.6	0.6	.7	0.4	0.4	0.4	0.6	0.9	0.3	0.3	0.1	0.5	1.1	0.5	0.5	0.5	0.6	0.58
9th.day	1.4	1.5	1.3	1.6	1.5	1.4	1.3	1.3	1.3	1.3	1.0	0.18	0.72	0.63	0.94	0.45	0.36	0.27	0.27	0.18	0.97
10th.day	0.4	0.2	0.75	0.6	0.8	0.6	0.4	0.5	0.4	0.4	1.5	1.0	1.0	1.0	1.0	1.0	1.1	1.2	0.93	1.22	0.87
11th.day	0.9	0.2	0.35	0.30	0.1	0.3	0.5	0.4	0.5	0.5	0.3	0.49	0.29	0.29	0.29	0.29	0.38	0.27	0.21	0.10	0.34
12th.day	1.0	1.0	1.0	0.11	0.8	0.6	0.1	0.3	0.5	0.3	0.3	0.1	0.24	0.24	0.24	0.24	0.24	0.24	0.30	0.12	0.39
13th.day	0.3	1.1	1.3	1.39	0.7	0.9	1.4	0.4	0.11	0.14	0.5	0.46	0.4	0.44	0.83	0.81	0.79	0.77	0.75	0.73	0.72
14th.day	0.6	0.6	0.49	0.30	0.4	0.4	0.4	1.2	1.29	1.37	0.9	0.54	0.99	0.50	0.3	0.41	0.52	0.63	1.04	0.85	0.66

No. of animals: 20 **Mean ± SD 0.71± 0.22**

Table 5: Healing rate of Staphylococcus aureus infected wound in mice treated by cefotaxime.

Days of the treatment	Healing rate mm.																				Mean
1st. day	0.9	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.45	0.6	0.7	0.61
2nd.day	0.2	1.0	0.7	0.5	0.3	0.4	0.3	0.4	0.4	0.4	1.1	1.1	1.1	1.1	1.0	1.0	1.0	0.55	0.4	0.3	0.68
3rd.day	1.1	1.0	1.3	2.7	1.3	0.5	0.6	0.6	0.6	06	0.9	0.1	1.0	1.2	1.5	1.1	1.6	2.0	1.1	1.9	1.16
4th.day	0.3	0.1	0.5	0.1	0.4	0.5	0.5	0.7	0.7	0.7	0.7	0.2	0.2	0.2	0.2	0.3	1.2	0.7	1.8	1.1	0.53
5th.day	0.4	1.5	0.9	1.3	0.8	1.5	0.9	0.4	0.3	0.3	0.3	.9	0.2	0.6	0.6	0.6	0.1	0.3	0.3	0.3	0.62
6th.day	0.8	0.2	1.1	1.3	1.3	1.2	1.8	1.3	1.4	1.4	0.7	0.5	0.8	0.4	0.3	0.1	0.8	0.7	0.6	0.7	0.87
7th.day	0.1	0.1	0.2	0.2	0.5	0.3	0.2	0.2	0.3	0.3	0.8	1.4	0.6	0.5	0.6	1.6	1.1	0.8	0.9	0.5	0.56
8th.day	0.5	0.9	0.5	0.9	0.9	0.7	0.8	1.3	1.0	1.2	11	0.6	1.1	0.5	0.3	0.4	0.8	0.5	0.2	0.5	0.74
9th.day	0.5	0.4	0.4	0.4	0.8	0.3	0.5	0.7	0.9	9	1.1	0.5	0.3	0.5	0.6	0.0	0.2	0.5	0.5	0.5	0.52
10th.day	0.5	0.6	1.0	6	0.2	0.6	0.6	0.6	0.3	0.7	0.4	1.5	1.7	2.2	2.3	0.1	0.3	0.9	1.1	0.5	0.81
11th.day	0.5	0.4	0.2	0.4	0.3	0.7	0.7	0.8	0.3	0.7	0.6	.4	0.4	0.4	0.4	0.23	0.8	0.6	0.4	0.8	0.60
12th.day	0.9	0.6	0.8	0.5	0.5	0.5	0.4	0.2	0.3	.4	0.7	0.2	0.2	0.3	0.3	0.3	0.2	0.2	0.2	0.4	0.40
13th.day	0.2	0.2	0.1	0.2	0.4	0.3	0.4	0.2	0.8	1.1	0.3	0.1	0.1	0.1	0.5	0.2	0.6	0.6	0.8	0.8	0.50
14th. day	1.0	0.8	0.2	0.3	0.5	0.5	0.5	0.6	0.6	0.3	0.2	0.8	0.5	0.2	0.1	0.2	0.2	1.	0.1	0.2	0.43
15th. day	0.1	0.9	0.7	0.4	0.3	0.3	0.2	0.3	0.21	0.4	0.2	0.3	0.9	1.2	0.1	0.5	0.5	0.1	0.1	0.5	0.42

No. of animals: 20 **Mean ± SD 0.61± 0.19**

Table 6: Healing rate of Staphylococcus aureus infected wound in mice treated by povidone iodine.

		Rate of healing mm. Days												
		1st.	2nd.	3rd.	4th.	5th.	6th.	7th.	8th.	9th.	10t	11th.	12th.	13th.
Laser0.5min exposure	20	2.3	1.02	1.0	0.64	0.84	0.6	1.06	0.64	0.64	0.46	0.56	0.56	0.86 ± 049
Laser1.0min exposure	20	1.2	1.02	0.62	1.38	1.28	0.94	0.60	0.70	0.68	0.82	0.46	0.20	0.82 ± 0.35
Laser2.0min exposure	20	0.66	0.9	0.94	0.84	0.80	0.64	1.06	1.32	0.10	0.30	0.54	0.24	0.35	0.74 ± 0.32
Laser3.0min exposure	20	0.6	0.86	0.96	0.96	0.60	0.52	1.70	0.9	0.06	0.46	0.24	0.06	1.22	0.70 ± 0.46

*** The variation among groups were not significant**

Table7: Healing rate of Staphylococcus aureus infected wound in mice treated by different periods of exposure to laser radiation.

Days of the treatment	Healing rate mm.																			Mean
1st. day	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.11	1.2	1.4	1.6	1.7	1.8	1.10	1.12	1.14	1.36	0.99
2nd.day	1.1	0.2	0.2	0.2	0.3	0.6	1.5	1.8	0.6	0.7	0.98	0.78	0.07	0.17	0.73	0.82	0.70	0.78	0.66	0.44	0.66
3rd.day	0.6	1.5	0.4	0.7	0.6	0.2	0.5	0.4	1.0	1.0	0.6	.2	0.79	0.67	0.2	1.1	0.7	0.6	0.9	0.7	0.67
4th.day	1.1	0.9	1.0	1.6	1.1	1.4	0.8	0.6	0.5	1.2	0.5	50	0.6	1.6	0.5	.8	0.50	0.31	2.20	1.4	0.96
5th.day	0.8	1.10	1.1	1.7	1.0	1.1	0.7	0.7	0.5	0.7	1.7	1.9	1.5	1.5	1	1.3	1.5	1.	0.2	0.3	0.87
6th.day	1.0	1.5	1.9	1.3	1.5	1.3	0.3	0.7	0.5	0.4	0.4	1.1	0.5	0.5	0.5	0.6	0.4	0.9	1.9	0.8	0.70
7th.day	1.2	1.4	0.5	0.6	1.5	.8	0.5	0.7	0.8	0.9	0.2	0.4	2.0	0.5	2.5	1.9	1.6	0.3	0.9	1.5	1.02
8th.day	0.6	0.6	2.2	1.4	1.4	2.6	1.4	0.3	1.1	0.6	1.1	1.0	0.5	0.5	0.5	0.5	0.5	0.5	0.6	1.5	0.61
9th.day	1.0	1.5	2.1	2.2	0.9	0.4	1.3	0.5	0.9	1.7	1.0	1.0	1.5	0.5	0.5	0.3	1.2	.5	0.5	0.5	1.0
10th.day	1.0	0.3	0.7	0.9	0.6	0.8	0.2	1.0	1.9	0.6	0.3	0.8	0.5	0.5	0.5	1.0	0.2	1.5	0.5	0.5	0.71
11th.day	0.5	0.6	0.5	0.6	0.6	0.5	0.6	0.5	0.8	0.3	0.8	0.3	1.5	1.0	0.7	0.6	0.6	0.5	0.2	0.5	0.61
12th.day	0.5	0.3	0.2	0.1	0.1	0.5	0.4	0.2	0.2	0.3	0.5	0.6	0.2	2.0	0.5	0.7	0.8	0.5	0.2	0.1	0.42
13th.day	0.3	0.1	0.3	0.1	0.1	0.5	0.3	0.4	0.1	0.5	0.3	0.3	0.2	0.1	0.5	0.1	0.1	0.1	0.0	0.1	0.22
14th. Day	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.9	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.14
15th. Day	0.0	0.1	0.1	0.0	0.1	0.1	0.1	0.4	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.0	0.1	0.15
16th. Day	0.1	0.1	0.0	0.1	0.1	0.1	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.04

No. of animals: 20 Mean ± SD 0.61± 0.32

Table 8: healing of Rate Staphylococcus aureus infected untreated wound in mice.

Days of the treatment	Healing rate mm.																			Mean
1st. day	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	2.0	1.1	1.32	0.5	0.66	0.67	0.89
2nd.day	0.1	0.6	0.6	0.2	0.4	0.4	0.1	0.1	0.2	0.3	0.3	0.6	0.6	0.4	0.4	0.4	0.08	0.4	0.4	0.3	0.31
3rd.day	0.1	1.1	0.4	0.5	0.2	0.5	1.3	0.6	0.5	0.4	0.56	0.19	0.5	1.1	0.3	2.19	2.5	0.1	1.2	1.2	0.79
4th.day	2.2	1.0	1.5	1.8	1.8	0.5	0.9	0.4	0.5	1.2	1.4	0.7	0.5	1.5	1.0	1.0	1.9	2.5	1.1	0.9	1.21
5th.day	0.7	1.0	0.2	0.7	0.8	0.8	0.9	1.9	1.8	0.6	0.6	1.1	1.0	0.5	0.8	1.6	0.6	0.5	0.2	0.9	0.86
6th.day	1.0	0.7	0.5	0.4	1.0	1.0	1.0	0.3	0.3	0.8	0.8	1.0	0.1	0.2	0.1	0.4	0.4	0.5	0.9	0.3	0.58
7th.day	0.5	0.4	0.5	0.5	0.3	0.3	0.3	1.0	0.3	0.6	0.6	1.0	0.2	0.2	0.4	0.2	1.3	0.5	0.5	0.6	0.90
8th.day	0.5	0.1	0.6	0.5	0.1	0.8	0.6	0.6	0.5	0.5	0.7	1.5	0.9	0.5	0.5	1.5	0.8	0.7	0.5	0.4	0.65
9th.day	1.0	1.8	1.3	0.8	0.8	0.6	0.1	0.3	0.5	0.6	0.7	0.9	1.9	1.6	1.5	0.4	0.9	1.0	0.7	0.8	0.91
10th.day	1.7	0.4	1.2	1.7	0.1	0.5	0.3	0.3	0.5	0.4	0.6	0.6	0.6	0.5	0.4	0.2	0.4	2.0	1.0	0.4	0.55
11th.day	0.8	0.5	0.8	0.2	0.4	0.5	1.4	0.7	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1.0	0.6	0.9	0.8	0.7	0.64
12th. day	0.8	1.1	0.3	1.2	0.5	0.5	0.3	0.9	0.5	0.6	0.5	0.3	0.4	1.0	0.9	0.1	0.5	1.0	1.1	0.3	0.63
13th.day	0.3	0.3	1.0	0.3	1.0	0.5	0.3	0.8	0.9	0.5	0.4	0.7	0.5	0.5	0.3	0.5	0.4	0.4	2.0	0.26	0.61
14th. day	0.6	0.6	0.7	0.6	0.6	1.0	0.5	1.0	1.1	0.5	1.1	0.6	0.5	0.67	0.68	0.09	0.1	0.1	0.1	0.1	0.70

No. of animals: 20 Mean ± SD 0.73± 0.021

Table 9: Healing rate of uncontaminated untreated wound in mice

Treatments	Rate of healing mm./day Mean ± SD
Cefotaxime + povidone iodine + 0.5 min. laser exposure	2.50±0.23
Cefotaxime + 0.5 min. laser exposure	1.45±0.27
povidone iodine + 0.5 min. laser exposure	1.56±0.08
Cefotaxime + povidone iodine	0.74±0.33
Cefotaxime	0.71±0.22
povidone iodine	0.61±0.19
0.5 min. laser exposure	0.86±0.49
Staphylococcus aureus contaminated untreated wound	0.61±0.32
Uncontaminated untreated wound	0.73±0.21

Groups	Cefotaxime + 0.5 min. laser exposure	Povidone iodine + 0.5 min. laser exposure	povidone iodine + Cefotaxime	Cefotaxime	Povidne iodine	0.5 min. laser exposure	Staphylococcus aureus contamina-ted untreated wound	Uncontaminated untreated wound
Cefotaxime + povidone iodine + 0.5 min. laser exposure	0.01	0.01	0.001	0.001	0.001	0.001	0.001	0.001
Cefotaxime + 0.5 min. laser exposure		NS	0.01	0.01	0.01	0.05	0.01	0.01
povidone iodine + 0.5 min. laser exposure			0.001	0.001	0.001	0.01	0.001	0.001
Cefotaxime+ povidone iodine				NS	NS	NS	NS	NS
Cefotaxime					NS	NS	NS	NS
povidone iodine						NS	NS	NS
0.5 min. laser exposure							NS	NS
Staphylococcus aureus contaminated untreated wound								NS
Uncontaminated untreated wound								

Table 10: Comparison among the efficacy of different treatment on the healing rate of experimentally induced wound in mice levels of significancy among groups enhancement of the healing of Staphylococcus aureus infected wound could be attributed to the above.

Discussion

Bactericidal effected of the laser:

The interaction between laser and bacterial cell depends on the wave length of the light, the power output of the laser and exposure time (the energy input), the beam diameter, and whether laser is continuos or pulsed mode. Such factors detect whether effects will be photochemical, photothermal, photoablative, or photomechemical. Photochimicl effects generate free radicals and single oxygen with power density less than Wcm^{-2} and exposure time more than 10 seconds.

Photothermal effects cause denaturation of cell constituents and vaporization of the cell with power density between $10\text{-}0^6$ Wcm^{-2} and exposure time between 10^3-10 seconds . Photoabltive effects cause breaking of chemical binds with power density 10^3-10^{10} Wc^{-2} and exposure time between 10^8-10^7 second. Photochemical effects cause for mutian of plasma followed by its explosive dissipation and generation of shock waves with power density between 10^{10}- 10^{13} Wc^{-2} and exposure time 10^{-11}-10^{-8} second [17] Malik et.al [18] , described the lethal photosenitization of *S. aureus* using white light sources and heamatoporphyrin as a sensitizer .Szuminsky et al. found that the high – voltage pulsed current produced antibacterial action against *S. aureus* , Sp. aeruginosa, Klebsiella Spp. and E. coli[19], Wilson et al have shown that *S. aureus* can be killed by short term exposure to light from a 7.3 m W He- Ne- laser in the presence of Toluidine- blue O as an exogenous photosensitizer (70) .It was found that more than 99% of *S. aureus* suspension can be killed by short term exposure to light from a 11 mV gallium aluminum aresenid (GaAa) diode laser with aluminum disulphonated photoalocyanine as exogenous photosesitizer [20] . methicilin resistant *S. aureus* strains were killed by short term exposure 15^{20} second to light from a low – power He – Ne laser on the presence of low concentration (12.5mg/ml.) of roluidine blue O.(10).

When *S. aureus* and Ps. aeruginose exposed to Nd: YAQ laser they were killed at energy dose 600 J [21]. The teeth root canals inoculated with dark stain and exposed to Nd: YAQ laser of 3J for 15 seconds followed by a15 seconds recovery interval showed sterilization of the tow treated canals out of eight canals without thermal damage of the surrounding tissue [22]. Helicobacter pylori was killed by a low power laser in the presence of photosensitizer [23].Non pigmented bacteria were not affected by low laser light [24]. Appropriate photosensitizer can be tender transparent organism susceptible to killing by the low power laser light. Gram positive Sactina lutea, E.coli ans Ps. aeruginosa coils be killed by Ghe – Ne – laser light but after treatment with toludinine blue O [25] used harmatoporphyrin as photosensitizer and found that *S. aureus* and E. coil were killed by He – Ne – laser in combination with this photodnsitizer [26]. These findings showed that a short period of exposure to laser was efficient to kill the bacterial cell and the

photosensitizer is an essential combination to enhance the microbial killing effect of laser radiation. These results coils explain the highest effectiveness of 0.5 minute laser exposure and the highest effectiveness when laser used in combination with povidone iodine photosenistizer compared with other combinations. Furthermore povidone iodine is an iodophore, a complex of elemental iodine with a carried, 1-vinyl -2- pyttolodinone polymer, which provides increasing solubility of the iodine and sustained – release of the iodine. It exerted antibacterial effect alone and potentiate the antiloacteroal effects of laser [27,28]. Therefore povidone iodine acts as an antibacterial and photosensitizer.

Effect of laser on the pathogenicity of *S. aureus*:

Many biochemical targets could be attacked by the laser. Al-Edhami et al [24] found the UV-B-radiation inhibited protein synthesis [29]. Usviarsov et al[25] found that there is a suppressive action for magnetic laser ray on the persistence factors, antilysozyme and anti-interfron of *S. aureus* and N. gonorrhoee [30]. Yasin found that positive DANase *S. aureus* became negative after exposure to laser [31]. Moreover coagulase, entrotocin. Lenkocidin and exotoxin of *S. aureus* were inhabited by laser radiation [11] which clearly indicates that laser radiation inhibited many biochemical parameters essential for the pathogenicity of *S. aureus.*

Effect of laser on the sensitivity of *S. aureus*:

Yasin found that laser decreased the MIC of the antibiotict required for inhibition of *S. aureus*. MIC of ampicillin decreased from 1024 mg /ml to 2651014 mg /ml after laser exposure [30].

Effect of the laser on the sensitivity of the microorganisms could be attributed to the changes occurred on their structural unites, therefore the pattern of sensitivity of the microorganisms was completely changes, they became more sensitive after exposure to laser [11]. Ali CI [27] found that laser especially if it combined with photosensitizer increase the sensitivity of *S. aureus* to chlorampheincol, gentamicin, tetracycline, erythromycin, methicillin, nitrofurantion, clindamycin, trimethoprime, ceftazindine, streptomycin, and colistin[31].

Enhnacement of wound healing:

In previous studies, many authors showed that Nd: YAG, carbon dioxide, Erbiun: YAG and diode laser enhance wound healing [31]. Bruce Reis et al.[28] compare CO_2 healing of laser with iodine surgical scrub in the healing of psendomonas infected wounds on the rabbit, and on frequency of wound breakdown secondary to sepsis the best results were obtained by laser [31]. Taylor et al compare the Nd: YAG and high power diode laser and they found that the degree of inflammation and collagen production was similar for diode laser and Nd: YAG laser [31] Kandela et al.[30] found that wound healing rate was significantly stimulated in various phases of healing process by repeated exposure to low dose

laser radiation Many mechanisms were given to laser as wound healing stimulators. Kandel et al said that this effect attributed to quicker response of phagocytic cell and initiation of fibroblastic reaction and rapid re-epithelization induced by laser[30]. Bruce Reid said that exposed to laser appeared earlier with higher activity which enhance wound healing [32].

Therefore, we can conclude that the enhancement of wound healing in this study could be attributed to bactericidal effect of the laser, effect of the laser on viability and virulency of the bactericidal, increase the sensitivity of the bacteria and enhancement of biochemical events participated in wound healing.

References

1 - Foote, C.S. (1990). Chemical mechanisms of photodynamic action. Proc. Spie. Institute advanced Optical Technologies on photodynamic therapy, 156, 115-126.
2 - Henderson, B.W. (1992). Dougherty. How does photodynamic therapy work. Photochemistry and photobiology, 55, 1, 145-157.
3 - Goldman, LThe biomedical lasers technology and clinical applications (Springer Berlin, 1981).
4 - Letokhov, V.S. (1985).Laser biology and medicine, Nature , 316,325-330.
5 - Mester, E. ; Lundang, G. ; Sellga, M. ; Szende, B. and Tata, J.G. (1968). The stimulating effect of low power laser rays on biological system. Laser Rev. 1-3.
6 - Frohlich, H. (1985). Further evidence forcohernt excitation inhiological system, phys.Lett. 110: 450- 481.
7 - Blinowska, K.J. ; leeh, W. (1985). A cell membrane as possible site of Froehlich's coherent oscillations, phys, Lett , 10. A 124-126.
8 - Mestetr, E. et al. (1971). Effect of lasers rays on wound healing .The American Journal of surgery. 122, 532- 535.
9 - Wilson, M. (1995). Killing of methicillin- resistant Staphylococcus aureus by low laser light J.MED. Microbial , 12 , 62-66.
10 - Al-Jebouri, M.M. and Al-Obaidy, H.S. (1997). Providine iodine as a new lethal photosensitizer for killing of disinfectant- resistant staphylococcus aureus of wound by light from helium-neon-laser. Proceeding of the first scientific conference, Saddam University, Iqaq. 1: 239-250.
11 - Cowan, S.T. and Steel, K.J. (1965). Manual for identification of medical bacteria, London,Cambrigge University press .
12 - Russel, A.D. ; Furr, J.R. (1986). Susceptitlity of purine and lipopolysacharides deficient Strains of Escherichia coil to some antiseptics and disinfectants. J. Hosp. Infect. 8 47-56.
13 - Platt, R.A. (1984). An experimental evaluation of antiseptic wound

irrigation.J. Hospital,Infect, 5,182-188.
14 - T. Whitney, R.R. (1976). Characterization and aualititation of experimental surgical Wound infection used toevaluate Topical antibacterial Agents.Antibacterial Agents Chemotherapy, 38-44.
15 - Willran, H.W. ; Goods, T.H. (1977). Studies of wound healing in experimental diabetesMellitus. Journal Surgical Research, 22,221-227.
16 - Franzer, L. ; Chassemiffar, M. ; Lonnberg, B. ; Schultz, G. ; Tarpila, E. (1996). Stimulation of Protracted connective tissue repair in normal mice by transformingGrowth factor B. Scan J. Plast Reconstr H and Surg. 30, 267-273.
17 - William, H. ; Patricia, M. (1978). New Method for assessing epidermal wound healing . The Effects of Triamcnolne acetonide and polyethylene film occlusion. J. Investigative Dermatology, 71,382-384.
18 - Kovasks, R.B. and Mester, E. (1974). Stimulation of wound healing with laser beam in rate,. Experiente. 30, 1272-1276.
19 - Schultz, R.J. and Harry, G.P. (1986). Batericidal effects of neodymium :YAD laser invitor study. Laser in surgery and medicine , 6, 445-448.
20 - Ramskold, L.O. ; Fong, C.D. and Stomberg, T. (1997). Thermal effects and antibacterial properties of energy levels required to sterilize stained root with Nd, YAG laser J. Ended. 23,96-100.
21 - Nillson, C.E. ; Wilson, M. and Macorobert, A.J. (1996). The killing of Helicobarcter pylori by low power laser light in the presence of a photosensitizer J.Med. Microbiol. 44,245-252.
22 - Macmillan, J.D. ; Maxawell, W.A. ; Chichester, C.D. (1996). Lethal photosensitization of Microorganisms with light from continuos gas laser . Photochemistry ,Photobiology, 5,555-565.
23 - Takashi, P.K. ; Toups, H.J. and Freemberg, D.B. (1976). Irradiation of E.coil in the visible spectrum with atonable organ dye laser energy source.Applied Microbiology, 19,63-67.
24 - El-Adhami, W. ; Daly, S. and Stewart, P.R. (1994). Biochemical studies on the lethal effects of Solar and artificial UV radiation on *S. aureus* Arch. Microbiol. 161,82-87.
25 - Usviatsov, B. ; Kirilchev, A.I. And Voronia, LG. (1977). The suppressive action of a magnetic laser ray and of an electroloyloc solution of sioudum hypochlorite on the factor of causative agents persistence. Zh. Microbiol Epididemiol. Immunobiol. 4,102-105.
26 - Yasin, A.N. (1998). Effect of He/Ne laser Radiation and sodium Hypochlorite. Disinfectanton the killing of Disinfectant Resistant S, aureus isolated from the wound MSc thesis. University of Tikreet.
27 - Ali, C.I. (1999). The effect of laser photochemical agent and thermal stress on some virulence Ffactors of S, aureus isolated from wounds, Ms.c. thesis.

University of Tikreet.

28 - Bruce Reid, A. ; and Stranc, M.F. (1991). Healing of infected wounds following iodine scrub or CO_2 laser treatment. Laser in Surgery and Medicine, 11,1-6.

29 - Perez martiz & Band Davide (1998). Silvers-D-skin resurfacing of the face with the Eribium:YAG laser-Dermatol. Surge, Jun,24(6):653-8;658-9.

30 - Kandela Siham, A. ; and Kantarjian Aida, H. (1998). Laser light in Enhancing wound healing rate . Journal of Saddam University Vol.2(4),December, 157-163.

31 - Tyalor, D. ; Lee, S.S.A. ; Nord quist ; Robert Payton ; Mark, E. ; Dickey, D. Thoma ; and Bartels Kenneth, E. (1997). Comparison of a High power Diode laser with theNd. YAG Laser using in Situwound and Medicine, 21:248-54.

32 - Pogrel, M.A. ; Guntenhoner, M. and Stren, R. (1993). profile of hyaluronidase activity distenguishs, carbon dioxide laser from scalpl wound healing Ann. Surg. 217,196-200.