WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 7, 1855-1868. Research Article ISSN 2277– 7105

# PHOTOSTIMULATION OF WOUND HEALING AND HAIR GROWTH OF SWISS ALBINO MICE UTILIZING LOW POWER LASER

**Mohemid M Al-Jebouri[1]* and Balsam Y Al-Shakarji[2]**

[1]Department of Microbiology, College of Medicine, University of Tikrit, Tikrit, Iraq.

[2]Department of Microbiology, College of Veterenary Medicine, University of Mosul, Mosul, Iraq.

Article Received on
17 May 2015,

Revised on 08 June 2015,
Accepted on 29 June 2015

***Correspondence for Author**
**Dr. Mohemid Al-jebouri**
Department of Microbiology, College of Medicine, University of Tikrit, Tikrit, Iraq

## ABSTRACT

**Background**: Many studies have demonstrated a bactericidal effect of laser irradiation toward apportunistic bacteria. Laser is a device capable of producing a beam of light that consist of parallel waves. This tool as a new photochemotherapeutic is used directly and induced microbial killing of photosensitized organism pending *in vivo* study. As well as uses applications in various fields of biology and medicine like wound healing. **Methodology**: Swiss albino mice were taken from Institute of the Biological and Pharmaceutical Research Laboratory (Baghdad). Low power laser,photosensitizer and antibiotic were used for treatment of wounded mice and infected with *Pseudomonas aeruginosa*. **Results**: It was concluded that *Ps.aeruginosa* could be killed by low-power diode laser in combination with photochemical agents which considered the most effective and most suitable than other agents .Wound healing and hair regrowth were significantly enhanced utilizing low power laser,photosensitizer and selected antibiotic.**Conclusions**: Povidone –iodine at concentration 256 µg/ml was a good photosensitizer to sensitize *P.aeruginosa* isolates for killing by low – power diode laser light with different exposure time . The statistical analysis of wound healing according to the type of treatment showed significant differences ($P<0.0001$) using F-test . The effect of laser exposure on various animal groups was statistically significant also ($P<0.0001$) using F-test . There was a significant difference between exposed animal groups and control group and the p value was less than 0.0001 using T-test.The time needed to regrowth of shaved hair of the experimental animals was shortened to be half of the time needed for hair regrowth without exposure to laser ,photosensitizer and antibiotic.

**KEYWORDS:** mice,wound healing,hair growth,laser,photosensitizer,cefotaxime.

## 1. INTRODUCTION

## 2. MATERIALS AND METHODS

### Isolation and identification of Pseudomonas aeruginosa

The isolation and identification of *Ps.aeruginosa* were carried out following Al-Jebouri and Al-Shakarchi.[4]

### Animals

Swiss albino mice were taken from Institute of the Biological and Pharmaceutical Research Laboratory (Baghdad) and were kept in the animal house of the College of Medicine of Tikrit university .They were kept in plastic cages in nine groups of mice (5 animals each ) with feed pellets from Iba center (Baghdad ). The animals were kept at room temperature adjusted to 21˚C . They allowed food and water properly every day.[5]

### laser

The low – power diode laser with measured output of 5mW (laser Becaon , I. N.C Michigan , U.S.A ) was used in the present study . This tool was emitting light with a wavelength of 650 nm in a collimated beam and diameter 1.3 mm .

### Photosensitizer

Povidone-iodine ( Jordan ) was used with concentration of 256 µg/ml.[6]

### Infection of the wound with *Pseudomonas aeruginosa*

### Inoculated bacteria

An isolate of *Pseudomonas aeruginosa* from infected human wounds was selected for this *in vivo* study. The organism was grown overnight in nutrient broth at 37 ˚C for 18 hours. The bacterial suspension was diluted with sterile nutrient broth to proportion of $10^5$.

Swiss albino mice of 25 gm weight were anesthetized with ether.[7] The dorsum of each animal was shaved and depilated .The dorsum was steriled using 70% of alcohol[8] and one wound was made in the dorsal midline using metal template . Length of incision is one centimeter which extended down to the paniculus carnosus and the wounds were then ready for inoculation. The animal were divided into nine groups , each group consisted of five animals.

**Groups of animals**

**Group No.1**

This group was considered as control with healthy five animals.

**Group No.2**

This group consisted of five wounded and uninfected animals.

**Group No.3**

This group consisted of five infected animals. The wounds were infected by taking a drop of Pasteur pipette containing $10^5$ CFU *Ps.aeruginosa*.

**Group No.4**

This group consisted of five infected animals, the wounds were infected by taking a drop of Pasteur pipette containing $10^5$ CFU *P.aeruginosa* which flooded with povidone – iodine. A repetition of treatment was made on 3 rd, 5th, 7th and 9th days of infection.

**Group No.5**

This group consisted of five infected animals , the wounds were infected by taking a drop of Pasteur pipette containing $10^5$ CFU *Ps.aeruginosa* and irradiated for 0.5 minute ,1,2,3 and 4 minutes respectively. A repetition of treatment was made on 3 rd, 5th, 7th, and 9th days of infection .The radiation distance was 30 from the laser source to the animals.[9]

**Group No.6**

This group consisted of five infected animals , and the wounds were infected by taking a drop of Pasteur pipette containing 105 CFU *Ps.aeruginosa* and these animals were injected intramuscularly (IM) with 0.028 gm/kg body weight every day in 2 divided doses, indeed 0.014 gm/kg Bw every 12 hours with cefotaxime.

**Group No.7:** This group consisted of five infected animals , with *Ps.aeruginosa* were flooded with povidone – iodine and then injected intramuscularly (IM) with cefotaxime at the same doses used in the group No.6

**Group No. 8:** This group consisted of five wounded animals, the wound was infected with *Ps.aeruginosa* and flooded with photosensitizer povidone –iodine (PI) and irradiated for 0.5 , 1,2,3 and 4 minutes respectively. A reptition of treatment was made on 3rd,5th,7th, and 9$^{th}$ days of infection.

**Group No.9:** This group consisted of five infected animals , the infected wounds were flooded with photosensitizer povidone – iodine (PI) and these animals were injected intramuscularly (IM) with cefotaxime at the same doses used in the group No.6 and No.7 . Animals were irradiated for 0.5,1,2,3, and 4 minutes respectively. A repetition of treatment was made on 3 rd, 5th, 7th, and 9th days of infection.

**Bacteriological culturing of the infected wounds**

Bacteriological samples were taken from all animals after infection and after all exposures using sterile swabs then swabs were inoculated onto blood agar plates and incubated at 37 ˚C and at room temperature for 24 hours . The cultures were purified on appropriate media ( Pseudomonas selective agar media and nutrient agar ) .The selected suspected colonies of *Ps.aeruginosa* were purified twice on nutrient agar and incubated at 37 ˚C and at room temperature for 18 hours . The inoculated nutrient agar slants of *Ps.aeruginosa* were made and kept at 4 ˚C for further investigations . The wounds were examined every day and the clinical observations were recorded.

## 3. RESULTS

**Wound healing**

Table 1 shows the effect of laser irradiation on the size of wounds infected with *Ps.aeruginosa* compared to non – exposed wounds of mice . It was found that animal group No.2 (only with wound ) showed complete healing on the 17th day of the experiment while group No.3 (wound +*Pseudomonas aeruginosa* ) showed complete healing on the 21th day of infection. Group No.4 showed complete healing on the 19th day of animal pathogenecity testing. Two animals of group No.5 revealed healing of the wounds on the 17th day and two animals showed wound healing on the 15th day from the start experiment but the last one revealed healing on the 14th day. Three animals were dead on the third day of experiment start which belong to the group No.6 and the two remaining animals acquired completeaccording to the type of treatment showed significant differences ($P<0.0001$) using F-test . The effect of laser exposure on various animal groups was statistically significant also ($P<0.0001$) using F-test . There was a significant difference between exposed animal groups and control group and the p value was less than 0.0001 using T-test.

**Table 1. Effect of laser irradiation on the size of wounds infected with *Pseudomonas aeruginosa* compared to non - exposed wounds of mice.**

| | gROUPS OF ANIMALS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| tIME OF EXPOSURE ( DAYS ) | g2 | | | | | g3 | | | | | g4 | | | | | g5 | | | | | g6 | | | | | g7 | | | | | g8 | | | | | g9 | | | | |
| | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 |
| | te | | | | | te | | | | | te | | | | | te | | | | | te | | | | | te | | | | | te | | | | | TE | | | | |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 1 | 2 | 3 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 1 | 2 | 3 | 4 | 0.5 | 1 | 2 | 3 | 4 |
| | sow | | | | | sow | | | | | sow | | | | | sow | | | | | sow | | | | | sow | | | | | sow | | | | | sow | | | | |
| **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** |
| **2** | **0.9** | **0.9** | **0.9** | **0.9** | **0.9** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **1** | **0.8** | **0.9** | **0.8** | **0.9** | **1** | **0.9** | **1** | **1** | **1** | **1** | **1** | **0.8** | **0.8** | **1** | **1** | **0.9** | **0.9** | **0.8** | **0.9** | **0.8** | **0.8** | **1** | **0.9** | **0.9** | **0.9** |
| **3** | **0.7** | **0.8** | **0.8** | **0.8** | **0.8** | **1** | **1** | **1** | **D** | **D** | **0.9** | **0.9** | **0.9** | **0.9** | **D** | **0.7** | **0.8** | **0.8** | **0.9** | **1** | **0.8** | **0.8** | **D** | **D** | **D** | **0.9** | **0.7** | **0.7** | **0.9** | **D** | **0.8** | **0.8** | **0.7** | **0.8** | **0.7** | **0.7** | **D** | **0.9** | **0.8** | **0.8** |
| **4** | **0.7** | **0.7** | **0.7** | **0.8** | **0.8** | **0.9** | **0.9** | **0.9** | | | **0.9** | **0.9** | **0.9** | **0.9** | | **0.6** | **0.8** | **0.7** | **0.8** | **1** | **0.7** | **0.7** | | | | **0.8** | **0.7** | **0.7** | **0.9** | | **0.7** | **0.7** | **0.6** | **0.7** | **0.6** | **0.6** | | **0.7** | **0.7** | **0.7** |
| **5** | **0.6** | **0.6** | **0.6** | **0.7** | **0.7** | **0.8** | **0.9** | **0.9** | | | **0.7** | **0.6** | **0.6** | **0.7** | | **0.5** | **0.7** | **0.7** | **0.7** | **1** | **0.6** | **0.6** | | | | **0.7** | **0.6** | **0.6** | **0.9** | | **0.5** | **0.5** | **0.5** | **0.5** | **0.4** | **0.5** | | **0.6** | **0.4** | **0.4** |
| **6** | **0.5** | **0.6** | **0.6** | **0.6** | **0.6** | **0.7** | **0.8** | **0.8** | | | **0.7** | **0.6** | **0.6** | **0.7** | | **0.4** | **0.7** | **0.6** | **0.7** | **0.9** | **0.6** | **0.6** | | | | **0.6** | **0.5** | **0.5** | **0.8** | | **0.4** | **0.4** | **0.4** | **0.3** | **0.3** | **0.4** | | **0.4** | **0.3** | **0.3** |

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **7** | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.7 | 0.7 | 0.7 | | | 0.6 | 0.5 | 0.6 | 0.7 | | 0.4 | 0.6 | 0.5 | 0.7 | 0.8 | 0.6 | 0.6 | | | | 0.5 | 0.5 | 0.4 | 0.8 | | 0.4 | 0.4 | 0.4 | 0.3 | 0.3 | 0.3 | | 0.3 | 0.3 | 0.3 |
| **8** | 0.4 | 0.5 | 0.5 | 0.5 | 0.5 | 0.6 | 0.6 | 0.7 | | | 0.5 | 0.5 | 0.5 | 0.6 | | 0.3 | 0.5 | 0.4 | 0.6 | 0.7 | 0.5 | 0.6 | | | | 0.5 | 0.4 | 0.4 | 0.8 | | 0.4 | 0.3 | 0.3 | 0.2 | 0.2 | 0.2 | | 0.2 | 0.2 | 0.2 |
| **9** | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.5 | 0.5 | 0.6 | | | 0.4 | 0.4 | 0.5 | D | | 0.3 | 0.5 | 0.3 | 0.5 | 0.7 | 0.2 | 0.6 | | | | 0.5 | 0.3 | 0.3 | D | | 0.3 | 0.3 | 0.2 | 0.2 | 0.2 | 0.2 | | 0.2 | 0.1 | 0.2 |
| **10** | 0.4 | 0.3 | 0.3 | 0.4 | 0.4 | 0.4 | 0.4 | 0.5 | | | 0.3 | 0.4 | 0.4 | | | 0.3 | 0.4 | 0.3 | 0.4 | 0.6 | 0.2 | 0.5 | | | | 0.4 | 0.3 | 0.2 | | | 0.3 | 0.2 | 0.2 | 0.1 | 0.1 | 0.2 | | 0.1 | 0.1 | 0.1 |
| **11** | 0.3 | 0.3 | 0.3 | 0.4 | 0.4 | 0.3 | 0.4 | 0.4 | | | 0.3 | 0.3 | 0.3 | | | 0.2 | 0.4 | 0.3 | 0.3 | 0.5 | 0.2 | 0.4 | | | | 0.4 | 0.2 | 0.2 | | | 0.3 | 0.2 | 0.1 | 0.1 | 0.1 | 0.2 | | 0.1 | 0.1 | 0.1 |
| **12** | 0.3 | 0.2 | 0.2 | 0.3 | 0.3 | 0.2 | 0.3 | 0.3 | | | 0.3 | 0.3 | 0.3 | | | 0.1 | 0.3 | 0.3 | 0.2 | 0.5 | 0.1 | 0.4 | | | | 0.4 | 0.1 | 0.2 | | | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 0.2 | | 0.1 | 0.1 | 0.1 |
| **13** | 0.2 | 0.2 | 0.2 | 0.2 | 0.3 | 0.1 | 0.2 | 0.3 | | | 0.2 | 0.2 | 0.2 | | | 0.1 | 0.3 | 0.2 | 0.2 | 0.3 | 0.1 | 0.3 | | | | 0.3 | 0.1 | 0.1 | | | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | | 0.1 | C | 0.1 |
| **14** | 0.1 | 0.1 | 0.1 | 0.2 | 0.3 | 0.1 | 0.2 | 0.3 | | | 0.2 | 0.2 | 0.2 | | | C | 0.2 | 0.2 | 0.1 | 0.2 | 0.1 | 0.3 | | | | 0.2 | 0.1 | 0.1 | | | 0.1 | C | C | C | C | 0.1 | | C | | C |
| **15** | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.2 | | | 0.1 | 0.1 | 0.1 | | | | 0.1 | 0.2 | C | C | 0.1 | 0.3 | | | | 0.2 | 0.1 | 0.1 | | | C | | | | | 0.1 | | | | |
| **16** | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.2 | | | 0.1 | 0.1 | 0.1 | | | | 0.1 | 0.1 | | | 0.1 | 0.3 | | | | 0.2 | 0.1 | 0.1 | | | | | | | | C | | | | |

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **17** | **C** | **C** | **C** | **0.1** | **0.1** | **0.1** | **0.1** | **0.1** | | | **0.1** | **0.1** | **0.1** | | | | **C** | **C** | | | **C** | **0.2** | | | | **0.1** | **C** | **C** | | | | | | | | | | | | | |
| **18** | | | | **C** | **C** | **0.1** | **0.1** | **0.1** | | | **0.1** | **0.1** | **0.1** | | | | | | | | | **0.2** | | | | **0.1** | | | | | | | | | | | | | | | |
| **19** | | | | | | **0.1** | **0.1** | **0.1** | | | **C** | **C** | **C** | | | | | | | | | **0.1** | | | | **0.1** | | | | | | | | | | | | | | | |
| **20** | | | | | | **0.1** | **0.1** | **0.1** | | | | | | | | | | | | | | **C** | | | | **0.1** | | | | | | | | | | | | | | | |
| **21** | | | | | | **C** | **C** | **C** | | | | | | | | | | | | | | | | | | **0.1** | | | | | | | | | | | | | | | |
| **22** | | | | | | | | | | | | | | | | | | | | | | | | | | **0.1** | | | | | | | | | | | | | | | |
| **23** | | | | | | | | | | | | | | | | | | | | | | | | | | **C** | | | | | | | | | | | | | | | |

G1,Control; G2,Wound; G3,Wound+P.aeruginosa; G4,Wound+P.aeruginosa+Povidone-Iodine; G5,Wound+p.aeruginosa+laser; G6,Wound+P.aeruginosa+Cefotaxime; G7,Wound+P.aeruginosa+Povidone-Iodine+Cefotaxime; G8,Wound+P.aeruginosa+Povidone-Iodine+laser; G9,Wound+P.aeruginosa+Povidone-Iodine+laser+Cefotaxime; D , dead; C, Complete closure of wound; 0,Zero with out wound; SOW ,Size of wound(cm); TE, time of Exposure.

**Table2 : results of swab culturing obtained from animal groups infected with *pseudomonas aeruginosa* exposed to various combination of leaser irradiation and antimicrobial agents.**

| | | | Groups of animals (5 each) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Date** | **Type of exposure** | **Time of exposure** | **G 1** | **G 2** | **G 3** | **G 4** | **G 5** | **G 6** | **G 7** | **G 8** | **G 9** |
| **8-4-2002** | **ZT** | **0.5** | - | - | + | + | + | + | + | + | + |
| | | **1** | - | - | + | + | + | + | + | + | + |
| | | **2** | - | - | + | + | + | + | + | + | + |
| | | **3** | - | - | + | + | + | + | + | + | + |
| | | **4** | - | - | + | + | + | + | + | + | + |
| **10-4-2002** | **FE** | **0.5** | - | - | + | + | + | + | + | + | + |
| | | **1** | - | - | + | + | + | + | + | + | **d** |
| | | **2** | - | - | + | + | + | **d** | + | + | + |
| | | **3** | - | - | **d** | + | + | **d** | + | + | + |
| | | **4** | - | - | **d** | **d** | + | **d** | **d** | + | + |
| **12-4-2002** | **SE** | **0.5** | - | - | + | + | + | + | - | + | + |
| | | **1** | - | - | + | + | + | + | + | - | **d** |
| | | **2** | - | - | + | + | + | **d** | + | + | + |
| | | **3** | - | - | **d** | + | + | **d** | + | + | + |
| | | **4** | **d** | - | **d** | **d** | + | **d** | **d** | - | - |
| **14-4-2002** | **TE** | **0.5** | - | - | + | - | - | - | - | - | - |
| | | **1** | - | - | + | + | - | + | - | - | **d** |
| | | **2** | - | - | + | + | + | **d** | + | - | - |
| | | **3** | - | - | **d** | + | - | **d** | + | - | - |
| | | **4** | **d** | - | **d** | **d** | + | **d** | **d** | - | - |
| **16-4-2002** | **FE** | **0.5** | - | - | + | - | - | + | - | - | - |
| | | **1** | - | - | + | + | - | + | - | - | **d** |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **2** | **-** | **-** | **+** | **+** | **-** | **d** | **+** | **-** | **-** |
| | | **3** | **-** | **-** | **d** | **d** | **-** | **d** | **d** | **-** | **-** |
| | | **4** | **d** | **-** | **d** | **d** | **+** | **d** | **d** | **-** | **-** |
| **24-30/4/2002** | **Fth E** | | **-** | **-** | **-** | **-** | **-** | **-** | **-** | **-** | **-** |
| | | | **-** | **-** | **-** | **-** | **-** | **-** | **-** | **-** | **d** |
| | | | **-** | **-** | **-** | **d** | **-** | **d** | **-** | **-** | **-** |
| | | | **-** | **-** | **d** | **d** | **-** | **d** | **d** | **-** | **-** |
| | | | **d** | **-** | **d** | **d** | **-** | **d** | **d** | **-** | **-** |

ZT,zero time;FE,first exposure;SE,second exposure;TE,third exposure;FE,fourth exposure;Fth E,fifth exposure;s,survived;d,dead.

**Table 3. Survival patterns of experimental animal groups exposed to various combinations of laser and antimicrobial agents.**

| | | | **Group of animal (5 each )** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **DATE** | **TYPE OF EXPOSURE** | **TIME OF EXPOSURE** | **G1** | **G2** | **G3** | **G4** | **G5** | **G6** | **G7** | **G8** | **G9** |
| 8-4-2002 | ZT | 0.5 | S | S | S | S | S | S | S | S | S |
| | | 1 | S | S | S | S | S | S | S | S | S |
| | | 2 | S | S | S | S | S | S | S | S | S |
| | | 3 | S | S | S | S | S | S | S | S | S |
| | | 4 | S | S | S | S | S | S | S | S | S |
| 10-4-2002 | FE | 0.5 | S | S | S | S | S | S | S | S | S |
| | | 1 | S | S | S | S | S | S | S | S | d |
| | | 2 | S | S | S | S | S | d | S | S | S |
| | | 3 | S | S | d | S | S | d | S | S | S |
| | | 4 | S | S | d | d | S | d | d | S | S |
| 12-4-2002 | SE | 0.5 | S | S | S | S | S | S | S | S | S |
| | | 1 | S | S | S | S | S | S | S | S | d |
| | | 2 | S | S | S | S | S | d | S | S | S |
| | | 3 | S | S | d | S | S | d | S | S | S |
| | | 4 | d | S | d | d | S | d | d | S | S |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 14-4-2002 | TE | 0.5 | S | S | S | S | S | S | S | S | S |
| | | 1 | S | S | S | S | S | S | S | S | d |
| | | 2 | S | S | S | S | S | d | S | S | S |
| | | 3 | S | S | d | S | S | d | S | S | S |
| | | 4 | d | S | d | d | S | d | d | S | S |
| 16-4-2002 | FE | 0.5 | S | S | S | S | S | S | S | S | S |
| | | 1 | S | S | S | S | S | S | S | S | d |
| | | 2 | S | S | S | S | S | d | S | S | S |
| | | 3 | S | S | d | d | S | d | d | S | S |
| | | 4 | d | S | d | d | S | d | d | S | S |
| 24-30 / 4-2002 | Fth E | 0.5 | S | S | S | S | S | S | S | S | S |
| | | 1 | S | S | S | S | S | S | S | S | d |
| | | 2 | S | S | S | d | S | d | S | S | S |
| | | 3 | S | S | d | d | S | d | d | S | S |
| | | 4 | d | S | d | d | S | d | d | S | S |
| Total count of survived animals | | | 4 | 5 | 3 | 2 | 5 | 2 | 3 | 5 | 4 |

ZT,zero time;FE,first exposure;SE,second exposure;TE,third exposure;FE,fourth exposure;Fth E,fifth exposure;s,survived;d,dead.

**Photochemical stimulation of hair growth of animals**

The present study revealed that the time consumed to regrowth of shaved hair of the experimental animals was reduced to half.This conclusion was observed with animal group no.9.This group was treated with laser,providon-iodine and cefotaxime.Normally,it was found that needed for complete regrowth of shaved hair was 14 days ( animal group no.2) and the treated group no.9 revealed that the time required for complete growth of hair was 7 days only.

## 4. DISCUSSION

The present study demonstrated that povidone-iodine at concentration 256 μg/ml could sensitize *P.aeruginosa* isolates for killing by low- power diode laser with different exposure times (Table 3) ,as well as laser radiation as a proper method to accelerate wound healing (Table1) . In the present study ,it was demonstrated that animals treated with laser and povidone – iodine combination belong to the group No.8 and animal treated with laser, povidone-iodine and cefotaxime combination belong to the group No.9 were more affected because earlier decrease in wound surface of irradiated mice in 14th day from the start of exposure comparable with other groups of animal which did not expose to laser and photosensitizer .These results agreed with that of Jasim who found that animals treated with laser , povidone-iodine and cefotaxime combination were more affected than other groups exposed to other combinations.[10] Jasim also found the treatment of animals with laser,photosensitizer and cefotaxime enhanced the growth of shaved hair.Stimulation of hair growth utilizing laser was also noticed elsewhere.[2] Furthermore, group No.8 and No.9 demonstrated earlier decrease in wound surface of irradiated mice in the 14th day of exposure, while other groups completed healing in the delayed time e.g .on 23rd day from the start of exposure because the use of laser was acceraleted of wound repair.These results agreed with Peterman et al . who found with laser light ,the energy required for the breakdown of waste building blocks and the synthesis of new building blocks for wound closure can be provided more quickly and ligament of wound repair accelerated , these processes are helpful in postoperative wound healing as well.[11] One of the results demonstrated in the present study that animal groups No.3,4,6 and 7 showed a delayed wound healing when non-exposed to laser because of the production of exotoxin A by *Ps.aeruginosa* and it is role in retardation of wound healing .These results agreed with that of Heggers et al. who founds that exotoxin A is the major mediator for retardation of wound healing in *Ps.aeruginosa* wound infections.[12] The present study showed delayed of wound healing in the animal group No.5 which treated with laser only without usage of povidone-iodine as photosensitizer comparable with group No.8 which treated with laser and povidone-iodine combination and group No.9 which treated with laser ,povidone-iodine and cefotaxime combination which might be due to the photodynamic therapy (PDT) which based on the dye-sensitized photooxidation of biological matter in target tissue.[13] This requires the presence of a dye (sensitizer) in the tissue to be treated.[14] Although such sensitizer can be naturally occurring as constituents of cells and tissue , in the case of PDT

they are introduced into organism as the first step of treatment .In the second step ,the tissue –localized sensitizer was exposed to light of wavelength appropriate for absorption by the sensitizer. These present results agreed with that of Burns et al. who found in the absence of the photosensitising agent , the laser light had no effect on the viability of the target organisms.[15] The present study also showed a delay in wound healing among the group No.4 which treated with povidone –iodine compared with other two groups (group No.8 and No.9) due to the usage of photosensitizer alone which did not affect the microorganism. These results reported here were almost similar to those of Wilson et al . who found neither the light nor the dye,when used alone , had statistically significant effect on the *Staphylococcus sanguis.*[16] As well as in the present experimental study group No.6 which treated with cefotaxime showed a delayed wound healing comparable to group No.8 and No.9 which showed that *Ps.aeruginosa* was highly resistant to this antibiotic. These results agreed with Naoom who found *Ps.aeruginosa* appeared resistant to cefotaxime.[17] Also these results were in agreement with Al –Amear who reported that *Ps.aeruginosa* isolates were resistant to carbenicillin ,cefotaxime and gentamicin.[18] Finally,the present study showed that the histological examinations revealed no any side effect of laser or injury to the treated tissue. The present results agreed with Jasim who found the tissue after exposure of laser appeared normal (76). Also Filonenko et al. found that when used different monochromatic optical sources (laser, diode laser) for pain relief and wound healing acceleration that involve the irradiation of tissue with monochromatic light at intensities that did not cause thermal changes or ionization in tissue.[19] The same results were obtained by Hornung et al.[20] and Burca et al.[21] Moreover,Oron also found that diode laser was very safe.[2]

## 5. CONCLUSIONS

It was found that povidone –iodine were good photosensitizer at concentration 256 µg/ml with different exposure times to sensitive *Ps.aeruginosa* for killing by low-power diode laser light and there were synergistic effects between laser light and the photosensitizers. Exposure of *Ps.aeruginosa* isolates to low-power diode laser light in the presence of photosensitizer such as povidone- iodine was very effective and led to acceleration of wound healing .Shaved hair of animals needed half time to be regrown compared to shaved hair without using laser,photosensitizer( providon-iodine and selected antibiotic (cefotaxime).

**REFERENCES**


1. Elzaim HS, Chopra AK, Peterson JW,Goodheart R , Heggers JP. Generation of neutralizing antipeptide antibodies to the enzymatic domain of Pseudomonas aeruginosa exotoxin A. Infection and Immunity, 1998; 66(5): 2170 - 2179.
2. Szuminsky NJ, Albers AC, Unger P. Effect of narrow pulsed high voltages on bacterial viability, Phy 1994; 74: 660-667.
3. AL – Senawi TH. Effect of laser radiation on the wound healing, M. Sc. Thesis,) Baghdad University, 1985.
4. Al-Jebouri M M and Al-Shakarjji B Y .The effect of low-power laser combined with providone-iodine photosensitizer on elastase production of Pseudomonas aeruginosa isolated from wounds.J Appl Med Sci, 2013; 2(2): 63-67.
5. Meripleg R, Whitney RR. Characterization and analititation of experimental surgical wound infection used to evaluate topical antibacterial agents. Antimicrobial agents Chemother, 1976; 38-44.
6. AL- Jebouri MM and AL – Obaidy HS . Povidine – 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, Iraq 1997; 1: 239–250.
7. Willran H, William H. Study of wound healing in experimental diabetes mellitus. J Surg Res, 1977; 22: 221-27.
8. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Win WC. Color atlas &text book of diagnostic microbiology. Fifth ed. Colorado U. S. A: 1997, p. 269.
9. Spirt ME.Effect of laser on wound healing. Bull. Socinchir 1973; 32:169-73.
10. Jasim TM. A study on the effect of laser irradiation on infectivity of Staphylococcus aureus and wound healing. PhD thesis, Tikrit University, 2000.
11. Ohiro T, Maeda T. Application of 830 nm diode laser lllt as successful adjinnkktive therapy of hypertrophoc scars and keloids. Laser therapy 1993; 155-66.
12. Heggers JP , Haydon S , Hayward PG , Carp S ,Robson MC . Pseudomonas aeruginosa exotoxin A : Its role in retardatin of wound healing : The 1992 Lindberg Award .J Burn care Rehabil,1992; 13(5): 512 – 18.
13. Wilson M .Photolysis of oral bacteria and its potential use in the treatment of caries and periodontal disease. J Appl Bacteriol, 1993; 75: 299-306.
14. Desimone NA, Chistiansen C, Dore D. Bactericidal effect of 0.95 –Mw helium –neon and

5 –mW Indium- Callium –Aluminum- Phosphate Laser irradiation at exposure times of 30, 60, and 120 second on photosensitized Staphylococcus aureus and Pseudomonas aeruginosa in vitro . J Ctaract Refract Surg, 2001; 27: 941 –47.

15. Burns T, Wilson M, Pearson GJ. Sensitisation of cariogenic bacteria to killing by light from a helium – neon laser. J. Med. Microbiol, 1993; 38: 401- 5.
16. Wilson M, Dobson J, Hervey W. Sensitization of Streptococcs sanguis to killing by light from a Helium / Neon laser. Oral Microbiol Immunal, 1993; 8: 182 – 87.
17. Naoom Y M .The effect of laser irradiation and photochemical agents on disinfectant-resistant Pseudomonas aeruginosa isolated from wounds. M. Sc. thesis, Tikrit University, 2000.
18. Al-Amir LA.Molecular study of virulence factors in Pseudomonas aeruginosa Ph. D. thesis, Baghdad University, 1998.
19. Filonenk N, Salansky N. Low energy photon therapy efficacy for the treatment of wounds and skin ulcers. abstract NAALT 2002.
20. Hornung R, Fehr M, Walt H,Wilson M. PEG-m-THPC-mediated photodynamic effects on normal rat tissues . Photochem Photobiol, 2000; 72: 696.
21. Burca c, Chimas A.Laser safty in medicin.Abstracts NAALT 2002.