Efficacy of Light-Emitting Diode Therapy on Diabetic Foot Ulcers

Heba A. Bahey El-Deen*, Adel A. Nossier** and, Mona M. Soliman***

Faculty of Physical Therapy, Misr University for Science and Technology. **Physical Therapy Department for Surgery, Faculty of Physical Therapy, Cairo University, ***Dermatology Department, National Institute of Laser Enhanced Sciences, Cairo University.

Abstract

The aim of this study was to determine the efficacy of light-emitting diode therapy in enhancement of chronic diabetic foot ulcer. Fifty patients participated in the study. They suffered from chronic foot ulcer as a complication of diabetes mellitus, their ages ranged from 50 to 70 years old. They classified into two equal groups (LED therapy group and standard medical therapy group). LED group received light – emitting diode therapy in addition to standard medical treatment for thirty sessions., while standard medical group received standard medical treatment only also for thirty sessions. Additionally two weeks period of follow up was added to the total program. There was statistical significant reduction in wound surface area after 15 and 30 sessions (P<0.05). There was also a statistical significant improvement in the degree of healing rate in LED therapy group through histopathological assessment of the tissue biopsy after 30 sessions. There were (16%) with fair healing, (76%) with moderate healing (8%) with excellent healing. While, in standard medical therapy group all patients (100%) were presented with poor healing. Therefore, light-emitting diode therapy is an effective, innovative, non expensive modality in the treatment of diabetic foot ulcer combined with standard medical treatment.

Key words: Light–Emitting Diode, Wound Healing, Diabetic Foot Ulcers.
INTRODUCTION

Diabetes mellitus is a major emerging clinical and public health in Egypt. The combined prevalence of diagnosed diabetics in Egyptian population was 9.3%. It is estimated that by the year 2025, nearly 9 million Egyptians (over 13%) of the population 20 years of age or older will have diabetes. 

Powell et al., (2004) mentioned that fifteen percent or more of people with diabetes sustain one or more foot wounds during their lifetime and they are fifteen times more likely to suffer from non-traumatic lower extremity amputation than people without diabetes. In addition, Margolis et al., (2002) mentioned that diabetic patients with lower extremity ulcers were hospitalized longer on average than those who were hospitalized and did not have ulcers whereas half of all lower extremity amputations in hospitalized patients occurred in diabetic patients. They also assured that those with lower extremity amputation have a diminished quality of life, increased health costs, and more likely to have the other limb amputated, and more likely to die within five years than those with no amputation.

Despite a great number of studies which have been conducted on acceleration of wound healing, delayed wound healing specially in diabetic ulcer is a continuing challenge in rehabilitation medicine. Many physical methods such as therapeutic ultrasound, laser therapy, and electrical stimulation were used for attainment of normal breaking strength and prevention of keloid and scar formation.

Ennis et al., 2007 stated that all mechanical or electrical modalities used for wound healing supposed to introduce energy to the affected area and activate certain processes inside the cell to trigger some sequence of reactions. It would be impossible to assume that one treatment or modality can generate all positive needed results. The future research should focus on helping clinician to decide which modality is optimum to use for how long and in what sequence to achieve complete healing in chronic diabetic wounds.

The need to care for a population with chronic wounds is a growing challenge that requires innovative approaches. The use of NASA light emitting diodes (LED) technology may enhance the natural wound healing process. This will save valuable time and resources for both patients and health care professionals. Furthermore, improved wound healing will reduce the risk of infection for the patient, decrease the amount of required costly dressings and more quick return of patient to a pre injury level of activity.

Whelan et al., in 2003 showed that the biochemical mechanism by which LED enhances the process of wound healing is not known. The current theory is that the infra red light is absorbed by some photoreceptors like hemoglobin, myoglobin, and cytochrome oxidase. LED treatment effectively energized the cells by stimulating their cytochrome oxidase and triggering cellular and molecular events that have significant biological benefits.
Al-Watban and Andres., 2003 used an array of 25 light-emitting diodes covering an area of 20 cm$^2$ to show its therapeutic effect on burn wound healing in both diabetic and non-diabetic rats. The study confirmed that light treatment enhances diabetic burn closure through its effect on microcirculation, beside the other known photo-biological effects. Those effects are increased cellular respiration and ATP synthesis in the mitochondria which fuels the metabolic repair processes. It also showed that the effect of polychromatic LED on burn wound closure was dose dependent. The doses 5 and 10 J/Cm$^2$ accelerated wound closure in both diabetic and non diabetic burn healing. These doses were incidental to the NASA LED dose of 7 J/Cm$^2$ which resulted in doubled formation of Nitric Oxide and collagen production during the first week of healing. The results showed that the diabetic rats appeared to benefit more from polychromatic LED treatment in burn healing than the non diabetic rats.  

This study was designed to evaluate the efficacy of using light-emitting diode therapy as a method of physical therapy approach on enhancement of healing of chronic diabetic foot ulcer was assessed by three methods of evaluative approaches.

MATERIALS AND METHODS

○ **Subjects:**
  ○ Fifty patients (44 males and 6 females) with type II diabetes mellitus and chronic full thickness (grade I) diabetic foot ulcers lasting longer than two months were recruited in the study.

**Criteria of Selecting Subjects:**

Patients participated in this study had the following criteria:

**Inclusive Criteria**

- Patients with type II diabetes mellitus and grade I (Full thickness) diabetic foot ulcer.
- Patient's age ranges from 50 to 70 years.
- Both sexes were included in the study.
- All patients have not been undertaken any prior physical modality for diabetic foot ulcer treatment.

**Exclusive Criteria**

- Patients who had any pathological conditions or associated injuries which may affect the results of the study.
- Patients who had skin disease or any disease which lead to ulcer other than diabetes as venous or arterial ulcers.
- Patients who presented with active malignancy.
- Patients who had any type of osteomyelitis associated with diabetic foot ulcer.
The patients were randomly divided into two groups:

(1) Group A (LED Therapy Group)

Twenty five patients received LED therapy for 30 sessions, three days per week in addition to standard medical treatment which given for diabetic foot patients.

(2) Group B (Standard Medical Therapy Group)

Twenty Five patients received standard medical treatment for 30 sessions. They were instructed to receive this treatment 3 times per week in diabetic foot clinic in Health Insurance Hospital, Nasr City.

Procedures:

a) Measuring Procedures:

1- Measurement of Wound Surface Area Using Digital Camera:

The digital camera was placed through a constant distance on a tripod from patient’s foot to capture a colored picture of ulcer to detect the size changes of the ulcer before treatment and at 15, and 30 sessions of treatment. The environmental conditions were controlled such as patient position, camera distance, and orientation and lighting level. A 20-Cm ruler was included in each photograph field to allow calibration during subsequent measurement procedures. The captured images were digitized to computer where surface area can be measured by specialized software program (Autovue Professional Ver 17 Build 7425, Cimmetry systems, Inc, USA).

2- Measurement of Wound Surface Area Procedure Using Transparency Film:

The patient was positioned in a comfortable position with exposure of the affected foot. Double sterilized transparent plastic films were placed directly flat and attached to the skin around the wound area with avoidance of any movement and distortion of the foot. Ulcer margins were traced by the same investigator to establish reliability of measurements. The traced ulcer margins was transmitted to be a digitized vector image by using a digitizer tablet and a stylus pen where the transparent film was placed flat on the digitizer tablet then the stylus delineated the margins of the traced wound. The digitized ulcer surface area was calculated by specialized software program (Autovue Professional Ver 17 Build 7425, Cimmetry systems, Inc, USA).

The mean of surface area has been calculated using both methods to ensure reliability of measurements. According to a study conducted by Krouskop et al 2002 it was found that there was a permissible error of ±3% of the calculated surface areas while Lagan et al 2000 found a significant difference in using raw tracing (24% larger than ) photographic method.
2- Pathological Assessment:

Tissue biopsy was obtained by a specialized dermatologist at the beginning and at the end of 30 sessions from each patient as follows:

☐ The ulcer area and adjacent skin should be sterilized by using Betadine Solution.

☐ When needed, the patient was injected sub-cutaneously with 0.5 ml xylocaine (local anesthesia) at the ulcer edge and adjacent areas.

☐ A sterile disposable punch biopsy was used to excise 4 mm\(^3\) skin biopsies from the edge and the center of the ulcer.

☐ Each specimen was put into a bottle containing 10% neutral formalin then sent to laboratory of histopathology to prepare each biopsy as prepared sections for staining.

Paraffin section technique:
- The specimen was fixed in 10% buffered formalin
- It was dehydrated in Alcohol and cleared in xylol solution.
- It was finally embedded into paraffin wax to form paraffin blocks.
- Each block was cut by microtome at 5 micron thickness and fixed on slides to be stained\(^2\).

- All sections were scored on a 0 to 4 scale for degree of epithelialization, cellular content, granulation tissue, collagen deposition and vascularity. The scoring was completed by a blinded evaluator who did not know about the fluence and frequency of the treatment. The blinded evaluator was a highly qualified individual with a specialty in histopathological analysis. The scores were added together to determine a total score which was ranged from 0 to 20 with degree of healing defined as follows: 0.0 to 5.0 poor healing, 5.1 to 10.0 fair healing, 10.1 to 15.0 moderate healing and 15.1 to 20.0, excellent healing\(^8\).

2) Therapeutic Procedures:

a- LED Treatment Protocol:

Application of LED therapy was given at power output of 50 mW and energy density of 4 J/cm\(^2\) for 8 minutes per session three times per week for 30 sessions. The probe was held vertically against each ulcer by direct contact with slight pressure to minimize power loss due to beam divergence\(^1\). A complementary 2 weeks follow up for each patient was added to the total duration of the study after completion of 30 session's program to show whether there is a continuing effect of light therapy on the healing ulcer.

b- Standard Medical Treatment Protocol:

a) Hypoglycemic medications such as insulin injection to control blood glucose level.

b) Systemic antibiotics against organisms according to culture tests.
c) Debridement: for removal of necrotic tissues and foreign bodies when needed.
d) Irrigation of the wound by normal saline twice daily.
e) Dressings: after irrigation of the ulcer. It was covered with sterile gauze.

**Statistical Procedures**

- In this study, the descriptive statistic was presented in the form of: mean, standard deviation, range, maximum and minimum values were calculated for all patients in all groups of the study.
- Paired "t test" was used for the "following up" within the same group of the study.
- Unpaired t-test was used to compare two groups.
- Chi square test determines how the frequency distribution for a sample fits the population.
- Mann-Whitney test was used to evaluate the difference between two populations using data from independent-measures.
- Wilcoxon test was used to evaluate the difference between two treatments using data from repeated measures of experiments.
- The data analysis and the level of significance were set at the 0.05 level.

P value > 0.05 insignificant
P < 0.05 significant

**RESULTS**

**Comparative Analysis of Data Between Both Groups in:**

**a- Demographic Data.**

As shown in Table (1), the mean difference was 1.5 in age while the mean difference of BMI was 0.4. Regarding to diabetes duration the mean difference was 1.9 while in the onset duration of diabetic foot ulcer was 0.3. The results of those previous data showed no statistical significance difference (P> 0.05) that could be detected between both groups of the study by using unpaired t-test.

<p>| Table (1): Comparison between Both Studied Groups as Regard to Demographic Data: |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Statistical Variables | Age (years) | BMI (Kg/m²) | Diabetes Duration | Onset duration of diabetic foot ulcer |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Group A | Group B | Group A | Group B | Group A | Group B | Group A | Group B |
| Mean | 58.2 | 56.7 | 28.4 | 28 | 15.5 | 13.6 | 3.7 | 4 |
| SD+ | 5.9 | 5.1 | 5 | 3.6 | 6 | 4 | 2.6 | 1.6 |
| Max. | 70 | | 41 | 36 | 32 | 25 | 8 | 6 |</p>
<table>
<thead>
<tr>
<th>Min.</th>
<th>50</th>
<th>20</th>
<th>21</th>
<th>5</th>
<th>5</th>
<th>2</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.Diff.</td>
<td>1.5</td>
<td>0.4</td>
<td>1.9</td>
<td>1.9</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T Value</td>
<td>0.8</td>
<td>0.09</td>
<td>1.9</td>
<td>0.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P Value</td>
<td>&gt;0.05 NS</td>
<td>&gt;0.05 NS</td>
<td>&gt;0.05 NS</td>
<td>&gt;0.05 NS</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

X = Mean, SD = Standard deviation
M. Diff. = Mean Difference
Max = Maximum, Min = Minimum
BMI = Body Mass Index.
Kg/m² = kilogram per meter square
Level of significance (P>0.05)
NS = Non significant
S=significant.
HS=highly significant.

### b- Measurements of Wound Surface Area:

The changes in surface area of diabetic foot ulcer in both groups before and after 15 sessions, 30 sessions and after two weeks of follow up period, a comparative data between both LED therapy group and standard medical therapy group was analyzed in Table (2) and Figure (1) from which it could be observed that wound surface area was smaller in LED group compared to standard medical therapy group starting from 15 sessions, until follow up. The results confirmed the use of the non-parametric Mann–Whitney Whillcoxon test to compare the values of both groups. The results of statistical analysis of this comparison showed that no statistically significant difference between groups before starting of treating sessions. While there was significant difference after 15 sessions and significant difference between groups after 30 sessions and two weeks of follow up period.

### Table (2): Comparison between Both Studied Groups as Regard Surface Area Changes before and After Treatment:

<table>
<thead>
<tr>
<th>Statistical Variables</th>
<th>Pre treatment</th>
<th>Wound Surface Area</th>
<th>Post Treatment</th>
<th>Post 15</th>
<th>Post 30</th>
<th>Follow Up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A</td>
<td>Group B</td>
<td>Group A</td>
<td>Group B</td>
<td>Group A</td>
<td>Group B</td>
</tr>
<tr>
<td>Mean</td>
<td>9.2</td>
<td>8.1</td>
<td>4.8</td>
<td>7.3</td>
<td>1.8</td>
<td>5.7</td>
</tr>
<tr>
<td>SD±</td>
<td>8.6</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>3.9</td>
</tr>
<tr>
<td>Z</td>
<td>0.6</td>
<td></td>
<td>5</td>
<td>5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>&gt;0.05</td>
<td>&lt;0.05</td>
<td></td>
<td>&lt;0.01</td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NS</td>
<td></td>
<td>S</td>
<td></td>
<td>HS</td>
<td></td>
<td>HS</td>
</tr>
</tbody>
</table>

Level of significance (P< 0.05)
NS = Non significance
S = Significance
HS = Highly significance
Figure (1): Comparison between the Mean Values of Wound surface Area in Both Studied Groups Before and After Treatment.

**c- Histopathological Changes:**

Table (3) and Figure (2) presented a comparative data analysis between both groups of the study as regard to histo-pathological changes before starting of sessions. The results revealed that there was no statistically significant difference could be detected between both groups of the study as regard different histo-pathological changes before starting of sessions by using un-paired t- test.

<table>
<thead>
<tr>
<th>Statistical Variables</th>
<th>Epithelialization</th>
<th>Cellular content</th>
<th>Granulation</th>
<th>Collagen</th>
<th>Vascularity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>1</td>
<td>0.5</td>
<td>1.3</td>
<td>1.3</td>
<td>1.4</td>
</tr>
<tr>
<td>SD+</td>
<td>0.7</td>
<td>0.4</td>
<td>0.6</td>
<td>0.6</td>
<td>1</td>
</tr>
<tr>
<td>T Value</td>
<td>1.9</td>
<td>0.02</td>
<td>1.4</td>
<td>0.09</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>P Value</td>
<td>NS</td>
<td>NS</td>
<td>&gt;0.05</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Level of significance (P> 0.05),
NS = non significant.
S=significant.
HS=highly significant.

![Bar chart showing mean values for different histopathological changes in LED Therapy Group and Standard Medical Therapy Group.]

Figure (2): Comparison Between Mean values in Both Groups as Regard to Histo-Pathological Changes Before Starting of Sessions.

Another comparative data analysis was shown in Table (4) and demonstrated by figure (3) between both groups as regard to histopathological changes after completion of sessions. One can observe that for vascularity, all histo-pathological changes were better in LED therapy group compared to standard medical therapy group. The results showed a significant difference between both groups by the un-paired t-test.

Table (4): Comparison between Both Groups as Regard Histo-Pathological Changes after Completion of Sessions:

<table>
<thead>
<tr>
<th>Statistical Variables</th>
<th>Epithilialization</th>
<th>Cellular content</th>
<th>Granulation</th>
<th>Collagen</th>
<th>Vascularity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>3.4</td>
<td>0.9</td>
<td>2.8</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>SD+</td>
<td>0.5</td>
<td>0.8</td>
<td>0.8</td>
<td>0.6</td>
<td>1.4</td>
</tr>
<tr>
<td>T Value</td>
<td>3.9</td>
<td>2.9</td>
<td>3</td>
<td>2.7</td>
<td>1.5</td>
</tr>
<tr>
<td>P Value</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
| Level of significance | (P> 0.05), | NS = non significant.
Figure (3): Comparison between the Mean Values of Both Groups as Regard Histo-Pathological Changes after Completion of Sessions.

The degree of healing rate after completion of sessions in both groups of the study was shown in Table (5) and demonstrated by Figure (4) in LED therapy group, there were 4 patients of (16%) with fair healing, 19 patients of (76%) with moderate healing and 2 patients of (8%) with excellent healing. While, in standard medical therapy group all patients 25 of (100%) were presented with poor healing. The results of this table showed a dramatic improvement in degree of healing rate in LED therapy group compared to non improvement after completion of session in standard medical therapy group with statistically significant difference in between by using chi-square test.

Table (5): Comparison Between Both Groups as Regard Degree of Healing After Completion of Treatment:

<table>
<thead>
<tr>
<th>Statistical Variables</th>
<th>Histo-pathological Changes</th>
<th>LED Therapy Group</th>
<th>Standard Medical Therapy Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Poor healing</td>
<td>Fair healing</td>
<td>Moderate healing</td>
</tr>
<tr>
<td>No</td>
<td>Group A</td>
<td>Group B</td>
<td>Group A</td>
</tr>
<tr>
<td>%</td>
<td>0</td>
<td>25</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>16%</td>
<td>76%</td>
</tr>
<tr>
<td>X^2</td>
<td>36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P Value</td>
<td>&lt;0.01 HS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Level of significance (P> 0.05),
NS = non significant,
S=significant. HS=highly significant.
Figure (4): Comparison Between Both Groups as Regard Degree of Healing After Completion of Treatment.
Sample Cases from LED Therapy Group Throughout the Treatment Program:

a- Before Treatment

b- After 15 Sessions of LED Therapy

c- After 30 Sessions of LED Therapy

d- After 2 Weeks of Follow up Period

Figure (5): Case 1 - Left Planter Ulcer Post Amputation of all Toes.
Figure (6): Case 3- Right Medial Ulcer at the Lower Third of the Leg.
Sample Cases from Standard Medical Therapy Group Through out the Treatment Program:

a- Before Treatment
b- After 15 Sessions of standard Medical Therapy
c- After 30 Sessions of standard Medical Therapy
d- After 2 Weeks of Follow up Period

Figure (7): Case 1- Right Planter Surface Ulcer.
Figure (8): Case 2- Right Planter Surface Heel Ulcer.

a-Before Treatment

b- After 15 Sessions of LED Therapy

c- After 30 Sessions of LED Therapy

d- After 2 Weeks of Follow up Period
Figure (9): Photomicrograph x20 with H&E staining obtained from a diabetic foot ulcer before starting LED therapy. Arrows show no epithelization, predominantly inflammatory cells, thick granulation formation, and extensive neovascularization.

Figure (10): Photomicrograph x20 with H&E staining showing a biopsy obtained from the same ulcer treated with LED therapy after completion of 30 sessions. Arrows show thick stratified squamous epithelium, fewer mature fibroblasts, and moderate neovascularization.
Figure (11): Photomicrograph x20 showing masson trichrome staining of the same previous ulcer before starting LED therapy. Arrows show few collagen bundles (A) compared to extensive collagen fibers (B) after completion of 30 sessions of LED therapy.
Figure (12): Photomicrograph x20 showing Periodic Acid Schiff staining of the same previous ulcer before starting LED therapy. Arrows show mild endothelial cell proliferation with mild basement membrane blood vessels (A) compared to extensive basement membrane vascularization (B) after completion of 30 sessions of LED therapy.
DISCUSSION

In the last few years, researches which were focused on the enhancement of wound healing mostly cover low level laser therapy studies, but the current availability of other light sources as light emitting diode invites research to explore its effectiveness as a possible alternative for low level laser therapy\textsuperscript{21}.

The current study used chronic diabetic foot ulcer that last longer than two months and this comes in agreement with the study of Smith, 2005 who stated that the magnitude of phototherapy effect depends on the physiological state of the cell at the moment of irradiation. For example when irradiating fresh wounds (acute) the irradiation effect can be minimal or non existent. This happens when cellular proliferation is active and the regeneration of the tissues is occurring at a more or less normal state while a great effect is observed for old (chronic) wounds. Therefore light will only stimulate cell proliferation if the cells are growing poorly at the time of irradiation\textsuperscript{17}.

The duration of the current study extended for 10 weeks as 3 sessions / week were given for LED group patients. This support the findings of Zimny et al., 2002 who conducted a study to estimate the appropriate duration of wound healing in patients with diabetic foot ulcer. He found a close relationship between the rate of healing and the underlying etiology of ulcer. They reported one to seven weeks as a minimal time for expected improvement of wound closure in diabetic foot ulcer\textsuperscript{25}. Despite it has been observed that, there was variation in closure rate of ulcer among LED group, as there were two ulcers closed after 18 sessions, 4 ulcers closed after 19 sessions, 1 after 20 sessions and 1 after 21 sessions. These may be attributed to the individual variations in response to phototherapy.

The complementary two weeks period of follow up was added to assure the efficacy of frequent regular visits of each patient even after complete healing to diabetic foot clinic. It was also used to monitor whether there is an extended effect of light therapy after completion of treating sessions or not. Findings of this current study ascertained this concept, as most of the ulcers in LED group further reduced or completely healed within this period of follow up. The study also, recommended an extension of this period to one or two months to show the proper duration length that the light effect was persisted. This followed Giurini and Lyons, 2005 who insisted on the importance of the care following complete healing of diabetic wounds for continued monitoring\textsuperscript{5}.

One of the most important findings of the current study was a highly significant improvement in the percentage of change in wound surface area within LED therapy group before treatment and after 15, 30 sessions and after two weeks period of follow up. There was a dramatic reduction in wound surface area throughout the treatment sessions. This comes in agreement with previous study reported by Vinck et al., 2003 who conducted a comparative trial between different wave lengths of LED on proliferation of chicken fibroblasts cultures. The authors proved the higher significant effect of all irradiated cellular cultures with LED compared to control non irradiated group. Consequently they revealed a higher significant effect of both green (570 nm) and red (660 nm) than infrared beam with 950 nm\textsuperscript{21}. 
The current study comes in agreement with some parameters and contradicts with others. The current study used both red LED at 670 nm and infrared LED at 950 nm that have been used while it did not use green LED at 570 nm.

Further more, the authors revealed that 0.2 J/Cm$^2$ as energy density had better effect than 0.9 J/Cm$^2$ or more. While the current study used 4 J/Cm$^2$ as an energy density. This contradiction may be attributed to the difference in nature between the irradiation of cellular culture environment as an invitro study and the irradiation of human skin as invivo study. Consequently, the higher increase in cell proliferation after irradiation with low wave length (570nm) in study of Vinck et al., 2003 may be due to neglected value of penetration depth because all wavelengths passed through a monolayer fibroblast cell culture.

Additionally, according to Van Breugel and Bar, 1992, absorption of light through fibroblasts was linked with several molecules who serve as photo receptors resulting in a number of absorption peaks as (420, 445, 560, 690 and 730 nm). Two wavelengths that were used in the current study (670nm and 730 nm) approached to those peaks.

Furthermore, longer wavelengths are more resistant to scattering than shorter ones. For example red light at 632.8 nm of He Ne laser penetrate 0.5-1mm before losing 37.5% of its intensity while longer wavelengths IR radiation will penetrate 2 mm before losing the same percent of its energy. All those previous findings come in agreement with the current study that used three long wave lengths which were (670nm, 730nm and 950 nm).

One of the most interesting results of the current study was the presence of highly significant difference and marked improvement of all scores and mean values of histopathological assessment before and after treatment within LED therapy group. It has been shown that all histopathological changes were better in LED group compared to standard medical therapy group after completion of sessions.

The current study revealed that 67% of patients in LED group presented with moderate healing rate as their scoring ranged from 10.1 to 15.0 according to Kawalek, 2004, and 8% of patients with excellent healing rate. These results showed significant difference in degree of healing rate in LED group compared to standard medical therapy group.

On the contrary of the current study which hypothesized that LED therapy has a beneficial effect in the increase of blood flow through vasodilatation and endothelial cell proliferation that were main factors to enhance healing of diabetic foot ulcer.

Pöntinen et al., 1996 compared the effect of laser light and light from LED source on head skin blood flow in 10 healthy men using laser Doppler technology. He concluded that laser therapy with wavelength 670 nm and given dose was ranged between 0.1 to 1.36 J/Cm$^2$ increased blood flow. While, the non coherent visible monochromatic irradiation from LED source with wavelength of 635 nm and given dose ranged from 0.68 to 1.36 J/Cm$^2$ decreased blood flow at least for 30 minutes after irradiation.
This comes in disagreement with the current study whose results revealed that there was a highly significant difference in mean values of vascularity and angiogenesis formation before treatment (1.5 ± 0.5) and after LED irradiation among LED therapy group (2.1 ± 0.6). However, it has been shown that there was non significant difference in mean values of vascularity and angiogenesis formation after completion of sessions between both study groups. As it was 2.1± 0.6 in LED group compared to 1.3± 0.6 in standard medical therapy group.

The marked improvement of wound healing in LED therapy group was attributed also to the reduction of bacterial growth and consequently infection of diabetic ulcer, this finding confirm the study conducted by Nussbaum et al., 2002 who reported interactions between both wave length of light and radiant exposure with different bacterial species. They concluded that, low intensity laser therapy (LILT) applied to wounds delivering commonly used wavelength of 630 nm and low radiant exposure from 1-4 J/Cm$^2$ could produce significant bacterial inhibition$^{13}$. Further more LED therapy group in the current study supposed to have the same advantage of bacterial photo inactivation through its administration of the same wavelength and the same radiant exposure.

Kawalek et al., 2004 who reported a scoring rate in wound healing dependant on summation of scores of histological findings of epithilization, granulation, cellular content, collagen deposition, and vascularity. The authors irradiated a group of diabetic mice with 980 nm Ga Al As diode laser with energy density 18J/Cm$^2$ every two days and reported a 100% percentage of wound closure after 19 days compared to 40% closure in control non-irradiated group$^8$. These findings agreed with the results of the current study which revealed a highly significant difference P < 0.01 in histological changes before and after irradiation by LED, and in between LED group and standard medical therapy group.

Hode and Tunér, 2000 presented a trial to clarify the difference between low level laser therapy (LLLT) and light emitting diode therapy (LEDT). They concluded that when tissue is irradiated, laser light gives stronger effect than incoherent light as LED of the same wavelength and the same dose. The most important difference between LED's and lasers is the coherence. It has been said that the coherence is lost when laser light is scattered in tissue. They added that the non coherent light is useful therapy but less efficient than laser therapy and probably most efficient on superficial structures only$^7$.

It has gradually become obvious that no single wavelength can accomplish all reactions required in the wound healing process, whereas combinations have proved significantly more effective$^{15}$. These results agree with the current study which has been used combination of three wave lengths simultaneously.

Smith, 2005 demonstrated that the wavelength in phototherapy is more important than the coherence. Furthermore, there is no significant difference whether the light used to stimulate growth was generated by a laser or from non coherent light of the same wavelength from a filtered lamp. These results support the conclusion that lasers bio stimulation is not magical but it is the light with appropriate wavelength which may produce the biological effects. More and more papers are appearing in the
therapy literature using non-coherent light sources such as LEDs. In general they are less expensive than lasers\textsuperscript{17}.

Rubinov. , 2003 presented a trial to show the physical mechanisms of biological effects of coherent and non-coherent light, he hypothesized that the primary interaction of light with biological matter is purely photochemical in nature, the absorption of light through some photoreceptors starts a chain of biochemical reactions, and in this case coherence of light is not important. In deed high coherence of light i.e. (when the phase of oscillations in electromagnetic wave remains unchanged for a long time) may be important if the phase of electron oscillations in the excited substance by light is also kept unchanged long enough. This is not true in bio-molecules which contain tremendous number of atoms in which their interaction with each other leads to rapid change of electron oscillations phase and because of this reason high coherence of light is not needed and photochemical effects produced by coherent and non coherent light will be indistinguishable\textsuperscript{16}.

Tunér and Hode., 2007 added that the coherent light waves may result in interference between its different beams and this interference may cause what is called laser speckles\textsuperscript{19}.

Rubinov., 2003 defined laser speckles as a strong modulation of light intensity which appears at the surface of optically inhomogenous medium as in biological tissues. Such modulation is a result of interference of micro beams reflected and scattered by inhomogenous medium. This is formed only at illumination of an object with coherent light. Illumination with photodiodes of non coherent light such as LED does not cause speckles formation and the tissue appears illuminated uniformly. One of the most interesting points which should be discussed to reveal that laser therapy has a disadvantage like formation of laser speckles. It was one of laser characteristic due to its coherency. Laser speckles cause a higher intensity of light than surrounding environment. In this way, islands of light appear larger than cells which they surround\textsuperscript{16}.

To the author knowledge, the current study is the first of its type. It opens a new field for further research for the exact determination of which phototherapy approach to be used as well as its optimum parameters for which conditions.

**Conclusions:**

From the obtained results of this present study, it may be concluded that Light Emitting Diode Therapy has a significant effect in acceleration of diabetic foot ulcer healing by improving micro circulation and increasing transcutaneous oxygen saturation to the ulcer region which lead to a significant contribution in the improvement of the patient's functional level, the quality of life and solving a major economical problem that face team members of ulcer rehabilitation.


