# Low-Level Laser Therapy for Diabetic Foot Ulcers: A Review and Analysis of Clinical Studies

THIS RESEARCH SUBMITTED TO THE PHARMACEUTICS CHEMISTRY DEPARTMENT / COLLEGE OF PHARMACY / HAWLER MEDICAL UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF B.SC. OF PHARMACY


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2023-2024

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## List of abbreviations :

- **DFU :** Diabetic foot ulcer
- **LLLT :** low level laser therapy
- **CCO :** Cytochrome c oxidase
- **NO :** Nitric oxide
- **KTP laser :** potassium titanyl phosphate laser
- **totHb :** total hemoglobin
- **VLF / VF :** Very low frequency / low frequency
- **HTM :** Brand name
- **BTL :** Brand name
- **RCT :** Randomized controlled trial
- **SG :** study group
- **CG :** control group
- **WSA :** wound surface area
- **HVPC :** high voltage pulsed current
- **SWC :** standard wound care
- **HBO :** hyperbric oxygen therapy

## Abstract:

Diabetes mellitus presents severe complications such as diabetic foot ulcers (DFUs), necessitating urgent interventions to mitigate morbidity and mortality. Traditional approaches encompass various treatments aiming to promote wound healing, prevent complications, and preserve limb function. Low-level laser therapy (LLLT) emerges as a promising adjunctive treatment, leveraging its ability to reduce inflammation, enhance angiogenesis, and accelerate healing in DFUs.

**Objective:** the purpose of this study was to determine efficacy of low level laser therapy in treating diabetic foot ulcers , and determine wich parameters were most useful.

**Method:** The review article conducted a comprehensive analysis of 11 studies investigating the efficacy of Low-Level Laser Therapy (LLLT) in treating diabetic foot ulcers (DFUs). the inclusion criteria involved studies comparing LLLT with traditional or other treatments and providing data on DFU healing. A systematic search of eight databases yielded 92 records, which were narrowed down to 11 articles after screening for relevance. Data extraction included study characteristics, LLLT parameters, treatment outcomes, and adverse events. The risk of bias was assessed using Cochrane criteria.

**Result:** The analysis revealed that LLLT, particularly semiconductor diode lasers with wavelengths ranging from 632 to 680nm, power density of 4 to 10 $J/Cm^2$, and treatment frequency of at least three times weekly for a month, significantly reduced ulcer size, promoted granulation tissue formation, and provided pain relief. Studies with longer treatment durations and higher treatment frequencies showed higher complete healing rates. However, some studies highlighted the importance of adequate treatment duration, with shorter durations (<4 weeks) resulting in significant ulcer size reduction but insufficient for complete closure.

Additionally, LLLT showed superiority over other therapies such as pulsed electromagnetic field therapy and hyperbaric oxygen therapy in terms of wound healing. Pain relief was consistently reported across studies, although the effect varied depending on the nature and source of pain. While most studies reported positive outcomes with LLLT, some inconsistencies were noted, emphasizing the need for further research with larger sample sizes, longer follow-up periods, and precise application methods.

**Conclusion:** the review underscores the effectiveness and safety of LLLT in DFU management, with benefits including ulcer size reduction, pain relief, granulation tissue formation, and promotion of complete healing. However, further high-quality studies are warranted to validate these findings and optimize treatment protocols.

# Chapter one ( Introduction into diabetes and lasers)

## 1/Introduction

Diabetes mellitus has a significant impact on global health, affecting approximately 422 million individuals worldwide and resulting in an estimated 2 million deaths annually. In the United States, it affects 11.3% of the population. Among the various complications associated with uncontrolled and prolonged diabetes, diabetic foot ulcer (DFU) is particularly debilitating and severe. DFU manifests as an ulceration, typically found on the plantar aspect of the foot (Raja et al., 2023) The high incidence of DFU and its associated mortality and morbidity make it one of the primary reasons for hospitalization among diabetes patients. During the early stages of diabetes, individuals may experience symptoms of foot sensitivity, such as pain and tingling. As the disease progresses, negative symptoms like numbness and weakness of the toes become more prevalent. Patients often exhibit a combination of pain sensitivity and dullness, along with decreased sensation and motor function in the limbs. These factors contribute to imbalance, unsteadiness, and an increased risk of falls. Furthermore, DFU is a leading cause of non-traumatic amputation and is associated with a higher risk of death due to the escalating morbidity (Wang et al., 2022). Numerous studies have been conducted to identify strategies that aid in ulcer healing and reduce morbidity and mortality rates. One such approach is low-level laser therapy (LLLT), also known as bio stimulation and photobiostimulation. LLLT involves the application of low-power monochromatic and coherent light to injuries and lesions, aiming to stimulate the process of wound healing (Houreld and Abrahamse, 2005). This therapy promotes the reduction of the inflammatory phase, facilitates angiogenesis, and enhances the production and organization of components within the extracellular matrix. Additionally, LLLT not only accelerates the healing process and reduces the size of the ulcer but also offers the advantage of easy administration. These benefits contribute to an improved quality of life for patients and minimize the impact of DFU.(Cardoso et al., 2021)

## 1.2 The Landscape of Diabetes Mellitus

In order to fully comprehend the seriousness of Diabetic Foot Ulcers (DFUs), it is crucial to have a comprehensive understanding of the different types of Diabetes Mellitus. Within the pancreas, specifically in the islets of Langerhans, there exist two primary subclasses of endocrine cells: beta cells, responsible for producing insulin, and alpha cells, which secrete glucagon. These beta and alpha cells continuously adjust their hormone secretions in response to the glucose levels in the body. However, when there is an imbalance between insulin and glucagon, the glucose levels become disproportionately skewed. In the case of Diabetes Mellitus, insulin is either absent or its action is impaired (known as insulin resistance), resulting in elevated blood sugar levels, or hyperglycemia.

1. **Type 1 Diabetes Mellitus (T1DM):**
   Type 1 diabetes mellitus is a medical condition characterized by the destruction of pancreatic β cells, resulting in the inability to produce insulin. This autoimmune disorder primarily affects children and adolescents, although it can manifest at any age. The autoimmune response triggers the presence of pancreatic autoantibodies, including islet cell autoantibodies and anti-insulin antibodies. Genetic predisposition, particularly associated with specific HLA-DR/DQ alleles, plays a significant role in the development of type 1 diabetes. Additionally, environmental factors such as viral infections, low levels of vitamin D, and early childhood nutrition contribute to its onset. Common symptoms of type 1 diabetes include increased thirst (polydipsia), frequent urination (polyuria), weight loss, fatigue, and heightened susceptibility to infections. In severe cases, individuals may experience diabetic ketoacidosis, a potentially life-threatening condition that requires immediate medical attention. The primary treatment for type 1 diabetes involves lifelong insulin therapy, as the body is unable to produce insulin naturally. It is worth noting that some patients may experience a temporary decrease in insulin requirements during a phase known as the "honeymoon phase" following diagnosis.

2. **Type 2 Diabetes Mellitus (T2DM):**
   Type 2 diabetes mellitus is the prevailing form of diabetes, characterized by the body's resistance to insulin and impaired secretion of insulin. It primarily affects adults, but there is a growing incidence among young individuals due to lifestyle changes that contribute to obesity and sedentary behavior. The combination of insulin resistance and dysfunction of β cells leads to elevated blood sugar levels.
   Unlike type 1 diabetes, type 2 diabetes is not primarily associated with autoimmunity, although there is a significant genetic predisposition. Environmental factors such as obesity, lack of physical activity, and unhealthy eating habits play a role in its development. Symptoms of type 2 diabetes may include increased thirst, frequent urination, fatigue, and delayed wound healing. However, the condition can remain undiagnosed for an extended period of time due to its gradual onset and mild symptoms.
   The management of type 2 diabetes involves making lifestyle modifications, including changes in diet, regular physical activity, and weight control. In some cases, oral medications and insulin therapy may be prescribed to regulate blood sugar levels, especially as the disease progresses.
3. **Gestational Diabetes Mellitus (GDM):**
   Gestational diabetes mellitus is a type of diabetes that occurs during pregnancy and is linked to a higher likelihood of complications for both the mother and the fetus. It is typically diagnosed through glucose screening tests conducted between the 24th and 28th weeks of pregnancy. GDM can result in unfavorable outcomes such as macrosomia (excessive birth weight), preeclampsia, premature birth, and the need for cesarean delivery. Several risk factors contribute to the development of GDM, including obesity, a family history of diabetes, advanced maternal age, and polycystic ovary syndrome. Treatment for

GDM usually involves making dietary adjustments, engaging in physical exercise, and, in certain cases, receiving insulin therapy to maintain blood glucose levels within the desired range. Women with GDM face an elevated risk of developing type 2 diabetes later in life, underscoring the significance of postpartum screening and ongoing management of diabetes.(Kharroubi and Darwish, 2015)

## 1.3 Diabetic foot ulcers

The estimated lifetime risk of foot ulcer development in patients with diabetes mellitus is 15%. Foot ulcers in these patients result in significant morbidity and are a notable risk factor for subsequent lower extremity amputations. Apart from reduced quality of life and higher healthcare expenses, individuals who undergo lower-extremity amputations often experience other concurrent medical conditions and have a higher likelihood of requiring amputation on the opposite limb. Furthermore, their mortality rate within the next 5 years is higher compared to individuals without amputations.(Kaviani et al., 2011)

This infection initially affects the surface layers of the body, but if treatment is postponed and the immune system is weakened, it can spread to the deeper tissues, leading to the development of gangrene and the need for amputations. Diabetic foot ulcers (DFUs) have a complex origin, influenced by various factors including socio-demographic elements like age, gender, place of residence, and educational background. Clinical factors such as the duration and type of diabetes mellitus (DM), inadequate control of blood sugar levels, increased body mass index (BMI), and foot deformities also contribute to the development of DFUs. Additionally, individuals with comorbidities such as peripheral vascular disease (PVD), retinopathy, nephropathy, and neuropathy are at a higher risk of developing DFUs. The delayed healing of wounds in diabetic foot cases can be attributed to vascular disease and neuropathy, as mentioned earlier. However, there are several other molecular factors that play a significant role in this process. In diabetes, there is a defect in wound angiogenesis, which refers to the formation of new blood vessels. Normally, wound healing involves a delicate balance between excessive and insufficient angiogenesis, but this balance is disrupted in diabetes. In diabetic wounds, there is a deficiency in angiogenesis, as evidenced by reduced blood vessel formation and prolonged time for wound closure.(Esmael et al., 2023)

## 1.4 Conventional treatment for DFU :

1. **Wound Dressings**: Wound dressings play a crucial role in the management of wounds, working in harmony with debridement. They assist in the removal of dead tissue, inhibit the growth of bacteria, regulate the flow of fluid from the wound, and create a moist environment that is free from infection. Although wound dressings alone have their limitations as the sole method of wound management, they make a significant contribution to the effective healing of wounds when used in conjunction with debridement. It is recommended to adopt a comprehensive approach by utilizing dressings along with

antimicrobial creams. For instance, hydrogels made from polymers such as chitosan and cellulose are commonly used. The dressing process aids in the development of granulation tissue and re epithelialization. In summary, the integration of debridement and dressing techniques is emphasized to achieve optimal wound care in chronic diabetic foot ulcers (DFUs).(Oyebode et al., 2023)

2. **Surgical Debridement:** Surgical debridement is a precise procedure that entails the thorough elimination of deceased and non-viable tissues from diabetic foot ulcers (DFUs). This meticulous process facilitates the establishment of a hygienic wound bed, which in turn fosters the formation of granulation tissue and re-epithelialization. Additionally, it diminishes the plantar pressures experienced in callused regions. The elimination of non-viable tissues holds paramount importance in terms of infection control, as these tissues can impede the flow of antibiotics and hinder the immune response. Sharp debridement is the preferred technique, and it is recommended to be performed every 24 to 72 hours in the presence of new necrotic tissue.
3. **Antibiotics**: The selection of antibiotic treatment is determined by the results of microbiological analysis and the presence of antibiotic resistance. When performing debridement, deep tissue cultures are collected to assist in choosing the appropriate antibiotics. In instances where superficial and stable diabetic foot ulcers (DFUs) do not show signs of infection, antibiotic therapy may not be required, and antiseptic wound dressings are typically adequate. For mild infections, oral antibiotics that specifically target the bacteria causing the infection are recommended. However, in cases of deep or limb-threatening infections, immediate administration of intravenous broad-spectrum antibiotics is necessary. It is generally recommended to administer antibiotics promptly and continue treatment for a duration of 14 days, which is typically effective.
4. **Vascular Assessment:** Peripheral arterial disease (PAD) has been found to have a significant impact on the healing of diabetic foot ulcers (DFUs) and can also lead to higher rates of amputation. Adequate blood flow is crucial in effectively combating severe infections associated with DFUs. The International Working Group on the Diabetic Foot (IWGDF) recommends urgent vascular intervention for patients who meet specific criteria, such as having low ankle pressure, toe pressure, ankle-brachial index, or transcutaneous oxygen pressure. In cases where there is extensive tissue loss or infection, revascularization may still be considered even if the pressure levels are higher. If wound healing does not occur within 4 to 6 weeks, despite optimal management, it is recommended to conduct further vascular imaging and consider revascularization as a treatment option.
5. **Offloading**: DFUs, or diabetic foot ulcers, are often caused by excessive shear stress and vertical pressure on the plantar region of the foot. To prevent and treat these ulcers, it is crucial to offload or alleviate and redistribute the pressure on the plantar area. The use of nonremovable knee-high offloading devices, such as total contact casts or knee-high orthoses, is highly recommended as a first-line treatment. These devices effectively redistribute the plantar pressure, thereby reducing the peak pressure experienced in the

forefoot. In cases where nonremovable devices are not well-tolerated, removable knee-high or ankle-high devices can be considered as an alternative. If nonsurgical offloading methods prove ineffective, surgical interventions like Achilles tendon lengthening or metatarsal head resection may be recommended.

6. **Amputation:** In certain cases of severe DFUs, despite attempts to salvage the foot, amputation may become unavoidable. The selection of the appropriate level of amputation is of utmost importance, as the amount of energy consumed after the procedure is inversely proportional to the length of the remaining limb. Whenever feasible, distal limb-conserving amputations are preferred in order to minimize the physical, financial, and emotional burden on the patient. It is crucial to effectively communicate to patients that positive outcomes can be attained following amputation, thanks to advancements in orthotics, prosthetics, and rehabilitation. The decision to proceed with amputation should be approached with caution, carefully considering the advantages and potential disadvantages. Regular monitoring and personalized care play a significant role in implementing a comprehensive approach to managing DFUs.(Kim et al., 2023)

## 1.5 Laser Therapy: A Novel Therapeutic Approach

### 1.5.1 Introduction and history

The emergence of low level laser therapy (LLLT), also referred to as photobiomodulation, can be traced back to the advent of the ruby laser in 1960 and the helium-neon (HeNe) laser in 1961. It was in 1967 that Endre Mester, while conducting research at Semmelweis University in Budapest, Hungary, made an intriguing observation. By applying laser light to the shaved backs of mice, Mester noticed that the hair grew back at a faster rate compared to the unshaved mice. Furthermore, he demonstrated that the HeNe laser had the ability to accelerate wound healing in mice. Building upon these findings, Mester extended his investigations to human patients, utilizing lasers to treat individuals with nonhealing skin ulcers. Over time, LLLT has evolved into a therapeutic procedure with three primary applications: reducing inflammation, edema, and chronic joint disorders; promoting the healing of wounds, deeper tissues, and nerves; and treating neurological disorders and pain.(Chung et al., 2012)

### 1.5.2 Cellular and tissue mechanisms of LLLT

At its most fundamental level, low-level laser therapy (LLLT) operates by initiating a photochemical reaction within the cell, a process known as biostimulation or photobiomodulation. When a photon of light is absorbed by a chromophore within the treated cells, an electron within the chromophore can become stimulated and transition from a lower-energy orbit to a higher-energy orbit. This stored energy can subsequently be utilized by the cellular system to carry out various tasks. LLLT primarily affects the mitochondria, leading to an augmentation in the production of adenosine triphosphate (ATP), modulation of reactive oxygen species (ROS), and the activation of transcription factors. Numerous transcription factors are regulated by alterations in the cellular redox state. These transcription factors then initiate the synthesis of proteins, which in turn trigger subsequent effects downstream, including heightened cell proliferation and migration, alterations in the levels of cytokines, growth factors, and inflammatory mediators, and an increase in tissue oxygenation.(Chung et al., 2012)

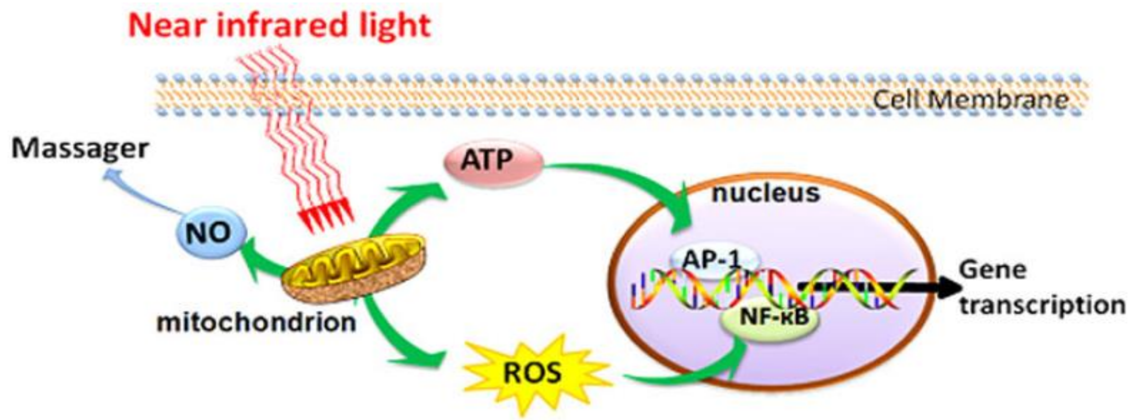


**Figure 1.1 Cellular mechanisms of low level laser therapy**

## 1.5.3 Laser Therapy Mechanisms: A Closer Look

The therapeutic benefits of laser therapy are primarily attributed to its ability to stimulate critical cellular processes. Key mechanisms of laser therapy in DFU management include:

1. **Effect on ATP**

PBM enhances mitochondrial activity, leading to a boost in adenosine triphosphate (ATP) synthesis, which serves as the primary energy source for various cellular processes. This augmentation in ATP production not only enhances the cells' ability to combat infections and expedite the healing process but also triggers the release of growth factors downstream. The interaction between growth factors and cell surface receptors initiates signaling pathways that transmit signals to the nucleus, prompting the transcription of genes responsible for increased cellular proliferation, viability, and migration. This effect is observed in a wide range of cell types, including stem cells and fibroblasts. When tissues are exposed to PBM, they absorb light of a specific wavelength through the enzyme present in the mitochondrial respiratory chain,(Esmael et al., 2023)

Cytochrome c oxidase (CCO) plays a pivotal role as the essential chromophore in the cellular response to low-level laser therapy (LLLT). CCO is a substantial protein complex located within the cell membrane, comprising two copper centers and two heme-iron centers. It serves as a crucial component of the respiratory electron transport chain. This chain facilitates the transfer of high-energy electrons from electron carriers through a sequence of transmembrane complexes, including CCO, until they reach the final electron acceptor. As a consequence, a proton gradient is generated, which subsequently contributes to the production of ATP. Consequently, the direct application of light has a profound impact on ATP production by influencing one of the transmembrane complexes within the electron transport chain. Specifically, LLLT leads to an augmentation in ATP production and electron transport..(Chung et al., 2012)

2. **Immune cells**

LLLT has a significant impact on immune cells, particularly on mast cells. Mast cells are crucial in the movement of leukocytes and play a vital role in inflammation. Certain wavelengths of light can stimulate mast cell degranulation, leading to the release of the pro-inflammatory cytokine TNF-a. This, in turn, results in an increased infiltration of leukocytes into the tissues. Additionally, LLLT promotes the proliferation, maturation, and motility of fibroblasts, as well as the production of basic fibroblast growth factor. Lymphocytes are activated and proliferate at a faster rate, while epithelial cells become more mobile, facilitating quicker wound closure. Furthermore, LLLT enhances the phagocytic abilities of macrophages.(Chung et al., 2012)

3. **Angiogenesis and Improved Microcirculation:**

LLLT has the potential to induce photodissociation of NO from CCO. The generation of NO by mitochondrial NO synthase (mtNOS), a specific isoform of NOS found in mitochondria, leads to the downregulation of cellular respiration as it binds to CCO and hinders its function. This binding of NO displaces oxygen from CCO, thereby inhibiting cellular respiration and subsequently reducing ATP production. However, through the dissociation of NO from CCO, LLLT effectively prevents this mechanism from occurring, leading to an enhancement in ATP production.(Chung et al., 2012)

The circulation is enhanced, inflammation is reduced, and the transportation of oxygen and immune cells through the tissue is improved by the release of NO, a powerful Vasodilator. The elevated level of NO may have had a significant impact on angiogenesis, as it is well-established that NO influences both angiogenesis and neovascularization.(Esmael et al., 2023)

LLLT has been demonstrated to induce vasodilation by initiating the relaxation of smooth muscle linked to the endothelium, which holds significant importance in addressing joint inflammation. Nitric oxide (NO) is a powerful vasodilator due to its impact on the production of cyclic guanine monophosphate. It has been postulated that LLLT may induce the photodissociation of NO, not only from CCO but also from intracellular reservoirs like nitrosylated versions of hemoglobin and myoglobin. This mechanism ultimately results in vasodilation.(Chung et al., 2012)

## 4. ROS production

The impact of low-level laser therapy (LLLT) on the electron transport chain goes beyond simply enhancing ATP levels in cells. The electron transport chain relies on oxygen as the final electron acceptor, which is then converted into water. During this process, a portion of the metabolized oxygen generates reactive oxygen species (ROS) as a natural by-product. ROS are chemically active molecules that play crucial roles in cell signaling, regulation of cell cycle progression, activation of enzymes, and synthesis of nucleic acids and proteins. Due to its ability to enhance oxygen metabolism, LLLT also promotes the production of ROS. Consequently, ROS triggers the activation of transcription factors, leading to the upregulation of various genes involved in stimulation and protection. These genes are likely associated with cellular proliferation, migration, and the production of cytokines and growth factors, all of which have been demonstrated to be stimulated by low-level light.(Chung et al., 2012)

Typically, ROS production is influenced by different wavelengths in a distinct manner. For instance, the application of 660nm laser light before or after an oxidative stimulus leads to an increase in ROS production. Notably, our research revealed that the 970nm laser light exhibited a moderate antioxidant effect, while a significant decrease in ROS levels was observed in cells exposed to either the 800nm laser light or a combination of the three different wavelengths.(Esmael et al., 2023)

## 5. Growth factors

Impaired wound healing in diabetic patients is often attributed to macro and microangiopathic effects that result in ischemia. The conventional wound healing process consists of several sequential phases, namely hemostasis, inflammation, proliferation, and remodeling. Growth factors, such as platelet-derived growth factor (PDGF) and transforming growth factor-beta (TGF-β), play a crucial role in this process. PDGF, which is released from platelet alpha-granules, attracts various cells like neutrophils, macrophages, and fibroblasts to the site of the wound. It acts as a potent mitogen, stimulating fibroblasts to produce new extracellular matrix components, particularly those that are non-collagenous in nature. On the other hand, TGF-β, released by different cell types, promotes collagen synthesis and reduces matrix degradation by fibroblasts. While TGF-β is essential for wound repair, it is also considered profibrotic and may contribute to increased fibrosis. The application of exogenous growth factors, including PDGF and TGF-β, has shown promise in accelerating the healing process. However, there are challenges that need to be addressed before their widespread use, such as effective application methods, development of suitable vehicles for delivery, and cost considerations.

Low Level Laser Therapy (LLLT) has been investigated for its potential impact on wound healing. Studies have indicated positive effects on cell migration, proliferation, and mitochondrial activity. Clinical trials, particularly those utilizing a 532 nm KTP laser, have demonstrated significant improvements in the healing of diabetic foot ulcers. Histopathological changes observed in these trials include increased thickness of fibrous tissue and activation of fibroblasts, accompanied by improved vascular activity.(Mousa et al., 2020)

**6. Pain Reduction**:

LLLT has the potential to elicit a biostimulatory impact on the nervous system, possibly due to its ability to enhance microcirculation in the affected region and promote nerve functional activity. This therapy may be particularly beneficial for individuals experiencing peripheral neuropathic pain, as it can facilitate the growth and myelinization of axons, ultimately aiding in the regeneration of damaged nerves. (Yamany and Sayed, 2012)

**7. Tissue Oxygenation and Autonomic Regulation Insights from Near-Infrared Spectroscopy Analysis:**

In a study conducted by (Salvi et al., 2017), the researchers aimed to investigate the effects of Low-Level Laser Therapy (LLLT) on diabetic foot ulcer (DFU) patients and healthy controls (HC). The LLLT treatment was administered using a device equipped with LED arrays that emitted light at wavelengths of 415, 633, and 830 nm. The subjects underwent a single session of LLLT in a controlled environment. To assess the response to treatment, the researchers monitored tissue oxygenation using near-infrared spectroscopy (NIRS) before and after therapy. The analysis of the data revealed significant changes in two key parameters: the concentration of total hemoglobin (totHb) and the ratio of very low-frequency to low-frequency (VLF/LF) oscillations in heart rate variability. For DFU patients, the study found a notable increase in totHb concentration following LLLT, indicating an improvement in blood flow to the affected tissues. Additionally, the VLF/LF ratio decreased significantly, suggesting a shift towards enhanced activity of the parasympathetic nervous system and reduced dominance of the sympathetic nervous system. These changes indicate a positive response to LLLT, which may potentially facilitate the wound healing processes. Interestingly, the intensity of the LLLT treatment seemed to have an impact on the magnitude of the increase in totHb concentration, with higher intensity configurations resulting in greater improvements. On the other hand, the HC subjects did not show significant changes in either totHb concentration or the VLF/LF ratio after the LLLT treatment. This suggests that healthy individuals have minimal physiological response to LLLT. These findings highlight the specificity of LLLT effects in targeting pathological conditions like DFUs, where improvements in tissue oxygenation and regulation of the autonomic nervous system are crucial for wound healing. Further research is necessary to gain a better understanding of the long-term effects of LLLT and to optimize treatment protocols for the management of DFUs.

### 1.5.4 Dosimetry (Laser-induced biological inhibition)

The response of cells to low-level light exposure, critical in wound healing, hinges on both the intensity and fluence of the light. Typically, low fluence levels stimulate cellular activity within a specific range, such as between 1 to 10 $J/Cm^2$ for in-vitro experiments, while higher fluence levels, exceeding 25 $J/Cm^2$, may trigger inhibition. This inhibitory effect is thought to be mediated by intracellular calcium (Ca) concentrations, which, when surpassing a certain threshold, activate intracellular nitric oxide synthase (NOS), leading to nitric oxide (NO) production. This NO production then slows down mitochondrial activity through various pathways.

In the context of wound healing, this inhibition of cellular activity due to elevated intracellular calcium and NO production can have significant implications. Wound healing involves complex cellular processes, including cell proliferation, migration, and extracellular matrix synthesis. High doses of light exposure, causing inhibition, could potentially impair these processes. For instance, inhibited cell proliferation and migration might delay wound closure, prolonging healing times. Additionally, reduced synthesis of extracellular matrix components could compromise the quality of the newly formed tissue.

Therefore, while low-level light therapy can be beneficial in promoting wound healing, with stimulatory effects observed within the 1 to 10 J/Cm$^2$ range, the use of higher doses should be approached cautiously. High doses of light exposure, leading to inhibition of cellular processes, may adversely affect wound healing outcomes. It's crucial to optimize treatment parameters to maximize therapeutic benefits while minimizing potential risks, particularly considering the critical thresholds for effective wound healing.(Tata and Waynant, 2011)

## 1.5.5 Photon-Tissue Interactions:

A thorough understanding of the complex interactions between photons and biological tissues is essential due to the intricate dance they perform. These interactions encompass various processes such as absorption, reflection, scattering, and transmission. Parameters like coherence, polarization, half-width full-maximum, and beam divergence play a crucial role in unraveling the intricate interplay that governs the clinical viability and effectiveness of PBM devices.(Mosca et al., 2019)

## 1.5.6 Chromophores and Light Penetration Dynamics:

The profound interplay between light and tissue is intricately woven together by key chromophores, namely melanin, hemoglobin (both oxyhemoglobin and deoxyhemoglobin), and water. The extent to which light can penetrate human skin is fundamentally linked to the absorption characteristics of these chromophores. The discovery of a specific range of wavelengths, known as the "optical window," within the red and near-infrared (NIR) spectrum (600–1070 nm) highlights the ideal dynamics for optimal tissue penetration.(Mosca et al., 2019)

## 1.5.7 Wavelength Optimization for Tissue Penetration:

The effectiveness of Low-Level Laser Therapy (LLLT) relies heavily on the concept of the "optical window" for specific wavelengths, especially in the red and near-infrared (NIR) spectrum ranging from 600 to 1070 nm. Within this range, tissue penetration is optimized due to the strong absorption properties of key chromophores (hemoglobin and melanin) at wavelengths below 600 nm. In LLLT applications, specific wavelength ranges are strategically utilized, such as 600-700 nm for superficial tissues and 780-950 nm for deeper-seated tissues.(Chung et al., 2012)

## 1.5.8 Exploration Beyond Traditional Wavelength Ranges:

Reports go beyond the traditional near-infrared (NIR) range and delve into the effectiveness of wavelengths in the near IR and mid-IR regions. This includes the utilization of carbon dioxide lasers (10.6 µm) and broad-band IR sources (10–50 µm). The presence of water as a chromophore, potentially in nanostructured forms within biological membranes, adds intricacy to comprehending responses that are specific to certain wavelengths.(Chung et al., 2012)

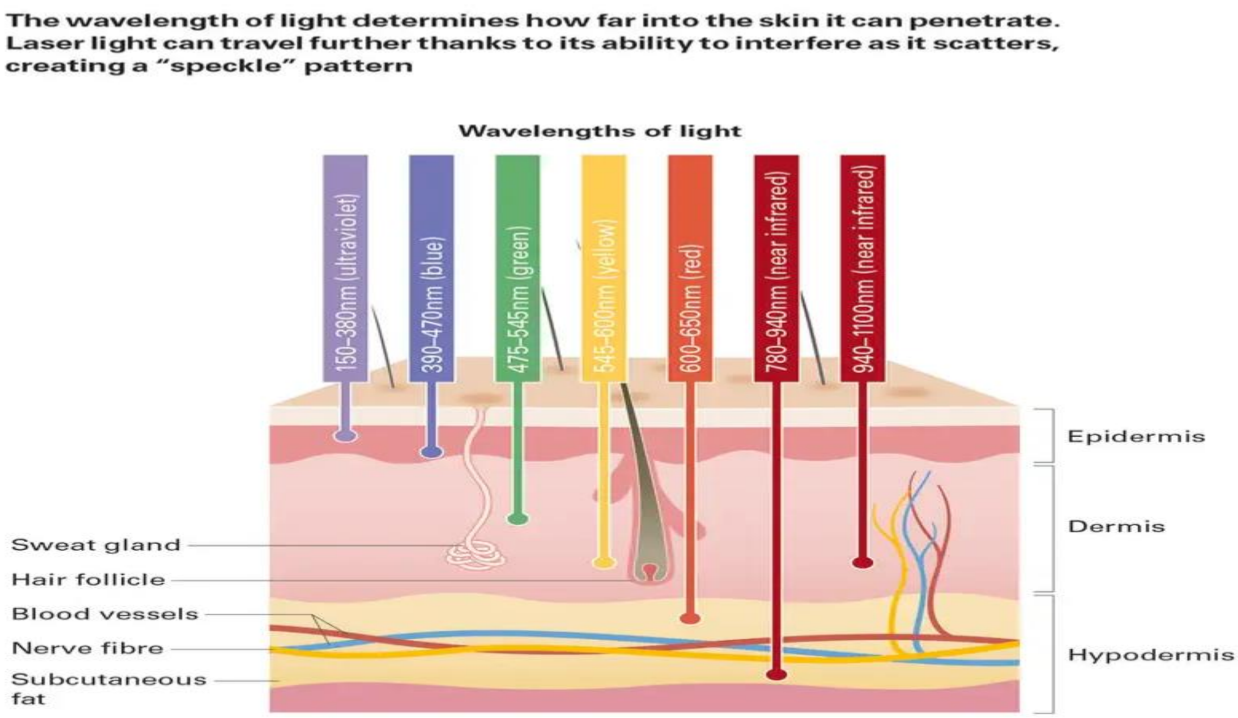


**Figure 1.2 different wavelengths of light penetration capacity into the skin**

# Chapter two

## 2/ Material and Methods

The present review article is aimed to investigate the effects of low level laser therapy on diabetic foot ulcers.

### 2.1 Inclusion criteria

Eligible studies had to meet at least one of the following criteria.

1. studies that explored the effect of LLLT on DFUs.
2. LLLT was compared with traditional or other treatments.
3. The study provided available results about DFU healing.

### 2.3 Exclusion criteria

Studies were excluded if they met either one of the following criteria.

1. Studies reporting the same sample; in this case, the most recent and most complete paper was chosen.
2. studies that were not human studies (i.e., vitro or animal).

### 2.4 Literature search

A systematic review was conducted on september 20, 2023 by searching eight databases, namely, PubMed, google schoolar, elsevier, NCBI, Hindawi, Springer, wiley, and scienceDirect, the search terms are as follows: “diabetic ulcer”, “diabetic foot”, “diabetic foot ulcer”, “foot ulcer” and “low-level light therapy”, “low-level laser therapy”, “LLLT”, “phototherapy”, and “laser”. References from these relevant studies were also reviewed to identify additional studies.

### 2.5 Data extraction

Data relating to the effects of LLLT on DFUs were extracted using a predetermined form and checked by the Author and supervisor, we extracted the following information from each included study: the first author's name, year, country, study design, demographic information, sample size, duration of diabetes, inclusion criteria, characteristics of the ulcers, LLLT parameters, treatment time, outcomes of treatment (i.e., complete healing rate, ulcer area reduction percentage and mean healing time), and adverse events if present.

### 2.6 Assessment of risk of bias and strength of evidence

Risk of bias in each of the included studies was assessed according to the Cochrane Handbook for Systematic Reviews of Interventions. Each study was assessed for the following six aspects: randomization generation, allocation concealment, blindness of participants and personnel, blindness of outcome assessment, incomplete outcome data, and selective reporting

## Chapter three (Results and discussion)

# 3/ Results

## 3.1 Search results

Initially, we obtained approximately 100 articles from our search. After removing any duplicate articles, we were left with a total of 92 records. Upon careful examination of the titles and abstracts, we excluded approximately 77 articles. Subsequently, we thoroughly reviewed the complete texts of 15 articles. Among these, four articles were excluded. This included two articles published in languages other than English, Portuguese, or Spanish, as well as two articles that did not meet the required criteria for inclusion. Ultimately, we included a total of 11 articles in our review and analysis, having completed the selection process.

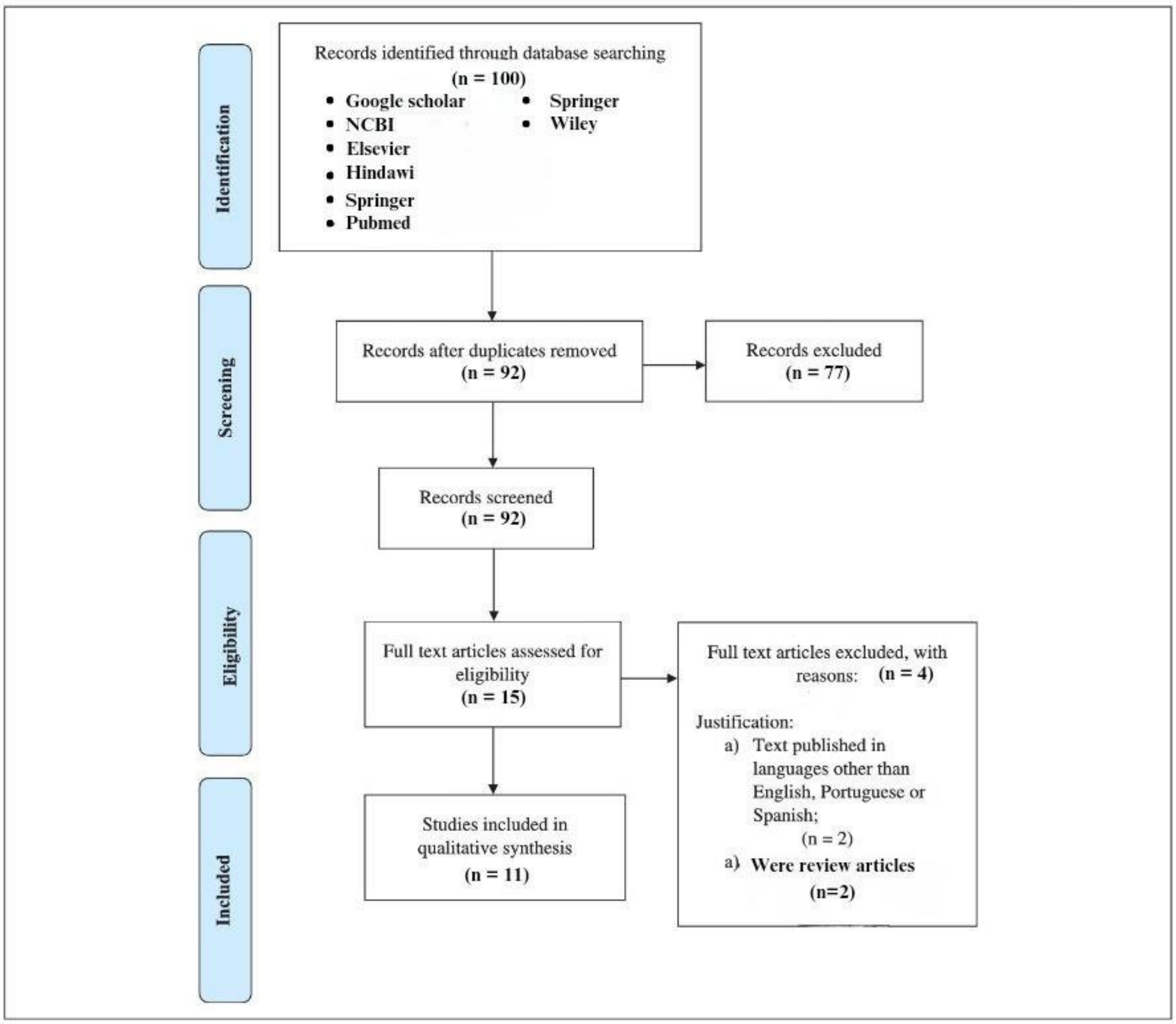


**Figure.3.1 Flowchart showing selection of studies included in this review.**

## 3.2 Characteristics of included studies

A total of 570 patients with diabetic foot ulcers (DFUs) were included in 11 studies published between 2011 and 2023. Among them, 300 patients received low level laser therapy (LLLT) combined with conventional treatment, while 270 patients received conventional treatment alone. The characteristics of these studies can be found in Table 1, while the laser parameters are presented in Table 2. It should be noted that not all information listed in the tables could be extracted from the included studies, and some data were missing possibly due to the specific aims and/or poor design of the studies. The number of patients in each study ranged from 16 to 164, and the study durations varied from one week to 20 weeks. The included studies utilized a range of LLLT parameters, including wavelengths of 532–980 nm, power densities of 4-400 mW, and fluences of 0.8–10 J/Cm$^2$. Among the eight studies comparing LLLT with conventional therapy, one study compared He-Ne laser with infrared laser, another study compared different wavelengths of infrared laser, and one study compared the effects of different energy densities. The outcomes assessed in these studies included ulcer area reduction, complete healing rate, ulcer healing time, ulcer granulation formation rate, and ulcer pain relief. Specifically, the outcomes of interest were the reduction in ulcer size and the rate of complete healing.

**Table.3.1 charectaristics of included studies**

| author | location / year | study duration | patient no | study group | control group |
|---|---|---|---|---|---|
| **Kaviani et al.** | Iran/ 2011 | 20 weeks | 23 | 13 | 10 |
| **Kajagar et al.** | India / 2011 | 15 days | 68 | 34 | 34 |
| **Feitosa et al** | Brazil / 2015 | 4 weeks | 16 | 8 | 8 |
| **MJ and EP** | India / 2018 | 15 days | 100 | 50 | 50 |
| **Tantawy et al** | UAE /2018 | 8 weeks | 65 | 33 | 32 |
| **Haze et al.** | Israel / 2021 | 12 weeks | 20 | 10 | 10 |
| **Darmaputri et al.** | Indonesia /2017 | 4 weeks | 28 | 14 -A (5J/Cm$^2$) | 14-B (10 J/Cm$^2$) |
| **Esmael et al** | Egypt / 2023 | 2 months | 45 | 15-15 (A+B) | 15 C |
| **Mousa et al.** | Iraq / 2020 | 1 week | 11 | 11 | 0 |
| **Mathur et al.** | India /2016 | 15 days | 30 | 15 | 15 |
| **Santhana Mariappan** | India / 2018 | 3 weeks | 164 | 82 | 82 |

**Table.3.2 laser charestaristics of the included studies**

| Author | laser type | wave length (nm) | power (mW) | energy density (jJ\Cm$^2$) | laser apllication time |
|---|---|---|---|---|---|
| **Kaviani et al.** | BTL | 685 | 50 | 10 | 200 sec \ 6 times per week for 2 successful weeks / then every other day |
| **Kajagar et al.** | thor international lid with multi diode cluster probe | 5 khz | 60 | 2 to 4 | daily |
| **Feitosa et al** | HTM | 632.8 | 30 | 4 | 80 sec /3 days per week |
| **MJ and EP** | red light | 660 | NA | 4 to 8 | daily for 20 min / for 15 days |
| **Tantawy et al** | He-Ne laser compared to Infrared laser | He-NEe632 \ INFL 904 | He-Ne 20 \ INFL 20 | He - Ne 5 \ INFL 6 | both lasers 90 sec per Cm$^2$ \ daily |
| **Haze et al.** | B-cure laser pulsed near infrared | 808 | 80 | 8.8 | 8 min per area |
| **Darmaputri et al.** | diode laser comparing 5 and 10 energy density | 830 | 400 | 5 and 10 | twice a week for 4 week |
| **Esmael et al** | infrared laser comparing wave length | 980-915-810-650 | 4 W | 4 to 10 | 2 sessions per week for 2 months |
| **Mousa et al.** | diode laser KTP | 532 | 8 W | 0.8 | 5 -10 min three times per week |
| **Mathur et al.** | diode laser | 660+-20 | 50 | 3 | 60 sec / daily |
| **Santhana Mariappan** | red laser | 635 | NA | NA | 30 min /daily |

## 3.3 Quality Assessment of the Studies

In the examined studies of this review, the prevailing unsatisfactory factors were the absence of a detailed account of allocation concealment, the lack of blinding among participants, professionals, and outcome assessors. Out of the total, three randomized controlled trials (RCTs) were conducted with a double-blind placebo-controlled design, while another three employed a randomized open label control study approach. Notably, all initially specified outcomes were duly reported. Additionally, one study provided information regarding the loss of patients. The synthesis of quality assessment results for each study is depicted in Figure 3.2

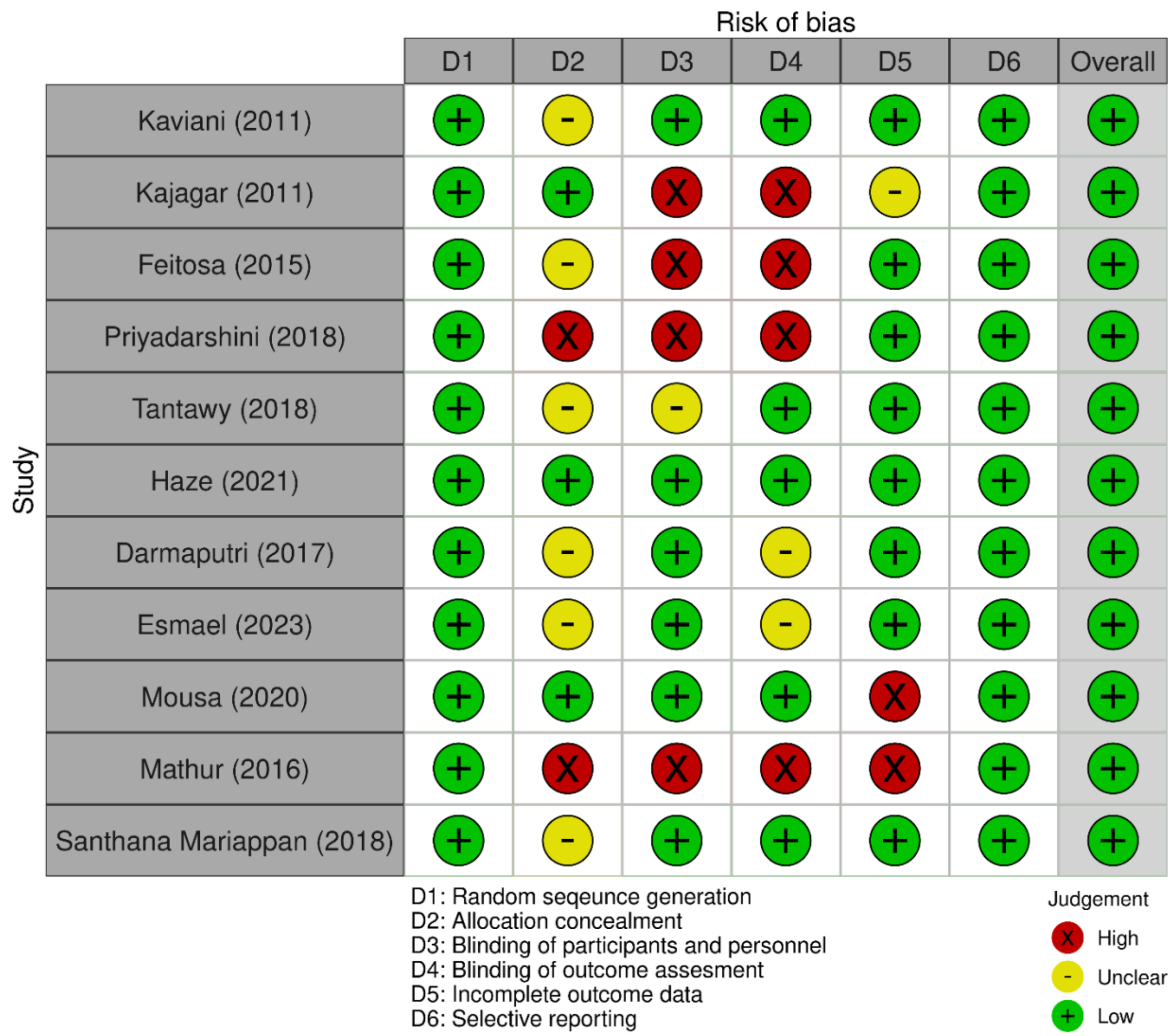


**Figure.3.2 Summary of bias risk assessment outcome for each study.**

### 3.4 Result of the includied studies

According to the studies Low level laser therapy showed a significant effect in reducing ulcer area and complete healing in patients with daibetic foot ulcer compared to conventional treatment. Conventional treatment usally consisit from :

**1. Wound care treatment**

a. Debridement to remove necrotic tissue. b. Irrigation of the wound by normal saline. c. Change dressing daily to protect the wound from infection. d.antibiotics according to culture e.slough excision

**2. Foot care**

a. Wash feet daily and dry them carefully, especially between the toes. b. Avoid extreme temperatures. c. Inspection daily of foot blisters.

**3. Footwear**

a. Avoid walking barefoot. b. Properly fitted shoes. c. Avoid wearing open-toed shoes.

**Table.3.3 Ulcer charectaristics of included studies**

| author | study duration | laser type | laser apllication time | initial ulcer area ( $Cm^2$) SG | initial ulcer area ($Cm^2$) CG | after treatment ulcer area ($Cm^2$) |
|---|---|---|---|---|---|---|
| **Kaviani et al.** | 20 weeks | BTL | 200 sec \ 6 times per week for 2 successful weeks / then every other day | 10.7+-25.7 | 7.8+-11 | ulcer area reduction SG :73.7+-10.2 % / CG :47.3+-15.4 % (after 4 weeks) |
| **Kajagar et al.** | 15 days | thor international lid with multi diode cluster probe | Daily | 26.08+-6.83 | 27.47+-6.03 | final ulcer area SG: 15.6479+-4.373 / CG: 24.2475+-5.5126 |
| **Feitosa et al** | 4 weeks | HTM | 80 sec /3 days per week | 7.98-+2.08 | 2.55-+0.77 | SG: 2.39+-1.26 / CG: 8.43+-1.84 |
| **MJ and EP** | 15 days | red light | daily for 20 min / for 15 days | 13.74-+11.88 | 19.09-+15.03 | SG :3.97-+5.41 / CG: 18.80-+17.70 |
| **Tantawy et al** | 8 weeks | HE-NE laser compared to | both lasers 90 sec per | G1 / 10.2 ± 5.6 | G2 / 9.5 ± 4.2 | G1: 3.7 ± 1.2 63.7% / G2: |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | Infrared laser | $Cm^2$ \ daily | | | 4.1 ± 1.3 56.8% |
| **Haze et al.** | 12 weeks | B-cure laser pulsed near infrared | 8 min per area | 12.4 ± 9.2 | 15.5 ± 17.1 | SG :1.5 ± 2.4/ CG: 12.5 ± 20.2 |
| **Darmaputri et al.** | 4 weeks | diode laser comparing 5 and 10 energy density | twice a week for 4 weeks | NA | NA | NA |
| **Esmael et al** | 2 months | infrared laser comparing wave length | 2 sessions per week for 2 months | A/ 19.9 ± 8.92 - B/15.95 ± 6.1 | C/ 12.79 ± 6.58 | A(2.17 ± 3.05) - B(2.85 ± 2.8) - C(9.76 ± 6) |
| **Mousa et al.** | 1 week | diode laser KTP | 5 -10 min three times per week | NA | NA | NA |
| **Mathur et al.** | 15 days | diode laser | 60 sec / daily | AVG 14.84 | AVG 13.52 | AVG SG: 9.3 / CG: 11.46 |
| **Santhana Mariappan** | 3 weeks | red laser | 30 min /daily | 26.256±6.6974 | 27.748±6.0339 | SG: 15.808±4.2612 / CG: 24.506±5.5232 |

In a study conducted by (Kaviani et al., 2011), a total of 23 patients were included, with 10 patients assigned to the placebo control group and 13 patients assigned to the low-level laser therapy (LLLT) study group. The ulcer surfaces of the patients were treated using a laser device (BTL, 685 nm, 50mW) at a fluence of 10 J/$Cm^2$, with an illumination time of 200 seconds. The laser device was used in noncontact mode, with a special head placed at a distance of 1 cm from the skin surface, resulting in an irradiation area of approximately 1 $Cm^2$ At the beginning of the study, the size of the ulcers was found to be greater in the LLLT group compared to the placebo group (10.7±25.7 cm2 vs. 7.8±11 $Cm^2$ but this difference was not statistically significant (p=0.799). The median number of LLLT sessions administered to the patients was 27, with a range of 12 to 54 sessions. After 2 weeks of treatment, the reduction in ulcer size was higher in the LLLT group compared to the placebo group (47.5±9% vs. 29.4±7.6%), although this difference was not statistically significant (p=0.125). However, after 4 weeks of treatment, the reduction in ulcer size in the LLLT group was significantly greater than in the placebo group (58±10.4% vs. 23.5±14.1%; p=0.046). At the end of the 20-week follow-up period, a total of 8 out of 13 ulcers (66.6%) in the LLLT group achieved complete healing, which was not significantly higher than the rate observed in the placebo group (3 out of 9 ulcers, 38.4%). The mean time for complete healing in the LLLT group was 11 weeks (95% CI, 7.3–14.7), which was lower than the mean time of 14 weeks (95% CI, 8.76–19.2) observed in the placebo group. It is worth noting that out of the 23 patients enrolled in the study, 5 patients were unable to complete the follow-up sessions until the 20-week mark.

Additionally, two patients from the placebo group required hospitalization and amputation due to extended gangrene. One patient in the LLLT group was hospitalized for treatment of infection. One patient from each group died due to myocardial infarction.

In another study by (Feitosa et al., 2015) ,The study involved 16 patients, divided equally into two groups of 8 each. The Low-Level Laser used in this research had a pulsed wave form, visible ray, and a wavelength of 632.8 nm. It had a peak potency of 30 mW and was referred to as Laser-HTM. The application time for the laser treatment was 80 seconds, which corresponded to an energy density of 4 $J/Cm^2$. The laser was applied directly to the wound without any physical contact, maintaining an approximate distance of 1 mm. The pen used for application was held perpendicular to the wound, and equidistant points around it were treated. The study lasted for a duration of 4 weeks.

After the 4-week period, both groups returned for re-evaluation. The results showed that the group receiving Low-Level Laser Therapy (LLLT) experienced a significant reduction in ulcer size and improved healing compared to the control group ($p<0.05$). In contrast, the control group exhibited an increase in ulcer size, and one patient even underwent a transfemoral amputation.

Furthermore, the study also assessed the pain levels experienced by the participants. The results indicated that the LLLT group demonstrated a substantial improvement in their pain levels on a scale of 1 to 10, with the average score decreasing from 9 to 5. Conversely, the control group did not experience any improvement in their pain levels.

**Figure 3.3 Ulcers submitted to the intervention with the low level laser, before and after.**

We have another study by (MJ and EP, 2018), This was a randomized open-label control study involving 100 patients, with 50 patients in the study group and 50 patients in the control group. The patients in the study group received treatment with Low-Level Laser Therapy (LLLT). Specifically, the ulcer bed with its edge was locally irradiated with red light at a wavelength of 660nm. The duration of exposure was calculated based on the size and depth of the ulcer, aiming to deliver a dosage of 4-8$J/Cm^2$ over a period of 20 minutes. This treatment was administered daily for 15 days. After the irradiation, conventional dressing was applied to cover the ulcer. In the control group, patients received conventional therapy alone, which included dressings with betadine or wet with saline, a course of antibiotic treatment, and removal of slough as necessary.

In the study group, out of the 50 subjects, 29 had grade 2 ulcers and 21 had grade 1 ulcers on day 1. At the end of the 15-day treatment period, none of the grade 2 ulcers remained in grade 2. Out of the 29 grade 2 ulcers, 28 (96.6%) improved to grade 1, and 1 ulcer was completely healed by day 15. Among the 21 grade 1 ulcers, 7 (33.33%) remained in grade 1, and 14 (66.67%) ulcers were completely healed.

In the control group, out of the 50 subjects, 26 had grade 2 ulcers and 24 had grade 1 ulcers on day 1. At the end of the 15-day period, 23 (88.46%) grade 2 ulcers remained in grade 2, and 3 ulcers (11.53%) improved to grade 1. Among the 24 grade 1 ulcers, all remained in grade 1, and none of the ulcers healed completely by day 15.

In another study by (Haze et al., 2022) wich is used in home photobiomodulation therapy in a frail population with severe comorbidities , This study was a single-center, prospective, randomized, double-blind, sham-controlled, parallel-group proof of concept study evaluating the safety and efficacy of a photobiomodulation device (B-Cure Laser, Good Energies, Haifa, Israel) at home for treatment of diabetic foot ulcers in addition to standard care. Patients with diabetic foot ulcers and comorbidities were randomized to receive active or sham treatments in addition to standard care Patients were treated everyday (excluding Saturdays) at home by professional caregivers until complete ulcer closure or up to 12 weeks, the earlier of the two. The light source was a pulsed near-infrared 808-nm Ga- Al-As laser, with a mean output power of 80 mW (peak power of 250 mW with a 33% duty cycle) and irradiation area of 4.5 × 1 $Cm^2$ which accumulates to 1.1 $J/Cm^2$ per minute. The device was applied by direct contact with the wound. Each application consisted of 8 minutes per area (total energy of 8.8 $J/Cm^2$), until all areas of the wound were irradiated. The part of the device that was in contact with the wound was cleaned thoroughly with 70% alcohol prior to application. the study included 20 patients ( 10 : 10) with the presence of osteo mylities in study group 9 of 10 patients and in control group 10 out of 10, with previous amputations in study group 6 of 10 and control group 5 of 10,all patienta had insulindependent diabetes type 2 for at least 10 years and Patients were all neuropathic (based on gross sensation test), mostly (18 of 20) with peripheral artery disease (based onpulses palpation and Doppler evaluation), All wounds were considered chronic showing no signs of healing with standard care for a minimum of 3 weeks. **(The wound charectaristics showed in table.3)**

After 12 weeks of treatment 7 of 10 patients achivied %90 closure and 5 patients achivied complete closure , in the control group only 1 patient achieved complete closure. primary wound treatment at the hospital, most patients with DFUs require frequent follow-up visits to the hospital clinic over a period of several weeks. This poses significant inconvenience with logistics that are often very challenging as well as safety issues at these times of social distancing and self-isolation of frail elderly patients with diabetes. The availability of a treatment that can be administered at home is therefore of great benefit.


**figure3.4 Diabetic foot ulcers submitted to active or sham photobiomodulatio (PBM) treatment at home (before/after). Upper panel: DFU of a patient in their early 70's, treated with active PBM. Laser device seen in the picture  Note near complete closure of the wound at 12 weeks. Lower panel: DFU of a patient in their 50's, treated with sham PBM. Note deterioration of wound by 8.5 weeks that led to transmetatarsal amputation**

In another study by (Darmaputri et al., 2020), The energy densities of 5 J/Cm$^2$ and 10 J/cm2 were compared in this study. The laser device used was a diode with a power output of 400 mW and a wavelength of 830 nm. The treatment was administered twice a week for a duration of 4 weeks. The sample size consisted of 28 patients, with 14 patients in group A receiving 5 J/Cm$^2$ and another 14 patients in group B receiving 10 J/Cm$^2$. All patients also received conventional treatment.

In group A, there was a significant difference in the median size of the diabetic foot ulcers (DFU) between before therapy and therapy at the end of week 4. However, from week 1 to week 3, there was a reduction in wound size compared to before treatment, but this reduction was not statistically significant.

In group B, there was a statistically significant decrease in wound size every week from the 1st to the 4th week. At the end of week 1, complete wound closure was achieved in 5 subjects (35.7%). By the end of week 3, 8 subjects (57.1%) had completed therapy as the wound closure was considered adequate.

It is important to note that incorrect parameters can decrease the effectiveness of therapy or even cause side effects. In this study, the unexpected result of non-significant reduction in wound size in group A may be attributed to an inappropriate dose. Therefore, careful parameter selection is crucial to achieve the optimal dosage for each application.

We have another study by (Esmael et al., 2023) In this study, the effectiveness of laser therapy in wound healing was investigated by comparing different treatment modes. The sample size consisted of 45 patients, divided into three groups: Group A (15 patients) received laser therapy in sequential mode using a combination of red and infrared wavelengths, Group B (15 patients) received laser therapy in separate mode, and Group C (15 patients) received conventional treatment.

The laser therapy was performed using the Summus, platinum P4, class laser therapy Diode laser, which is the first device capable of delivering a combination of four wavelengths (980, 915, 810, and 650) in the same beam. The laser therapy was administered in six phases during each session, with the auto parameter calculation ensuring synchronization of the four wavelengths based on the size of the wound. This comprehensive approach aimed to optimize the laser tissue interaction and promote proper wound healing. The study duration was two months, during which the patients underwent two sessions per week. The results, as shown in Table 3, revealed that Group A exhibited the highest reduction in ulcer area compared to Groups B and C. Additionally, in Group A, nine patients achieved complete wound closure, while in Group B, only four patients achieved the same outcome. Group C had the lowest number of patients (one) who achieved complete wound closure.

The combination of red and infrared lasers has been found to be effective in promoting the repair of skin wounds. These lasers induce the growth of fibroblasts, collagen synthesis, angiogenesis, and subsequent re-epithelialization, leading to wound closure. The study suggests that using red and infrared wavelengths in sequential mode provides a promising alternative for achieving optimal results in terms of wound closure percentage and the time required for complete wound healing. This is attributed to the activation of photoreceptors by the red laser, which enhances the absorption of infrared lasers and increases the production of ATP, a crucial component for wound repair.

We have another study done by (Tantawy et al., 2018) comparing the effects of Helium neon laser (HNLT) and infrared laser (ILT) on diabetic foot ulcers, a sample size of 65 individuals was divided into two groups. Group 1 consisted of 33 participants who received HNLT along with conventional treatment, while Group 2 comprised 32 individuals who received ILT along with conventional treatment. The study lasted for a duration of 8 weeks.

After 4 weeks, both the HNLT and ILT groups exhibited a statistically significant reduction in the surface area of the ulcers, with a p-value of less than 0.05. This reduction in ulcer surface area remained significant even after 8 weeks of treatment in both groups.

Upon comparing the two groups, it was observed that the reduction in ulcer area was slightly greater in Group 1 (HNLT + conventional treatment) compared to Group 2 (ILT + conventional treatment). However, this difference was not statistically significant, with a p-value greater than 0.05 (63.7% versus 56.8%).

Overall, the study findings indicate that both HNLT and ILT are valuable in the treatment of diabetic foot ulcers and are equally effective in accelerating the healing process of these ulcers.

## 3.5 complete ulcer closure

Complete ulcer closure was achivied In six studies with different duration and charectaristics as follws :

1. in the study done by {Kaviani, 2011) they used BTL laser In LLLT group, patients received illuminations over the ulcers, six times per week, for at least two successive weeks and then every other day up to complete healing ( $20^{th}$ week), In this study, the effects of Low-Level Laser Therapy (LLLT) on ulcer size reduction were examined compared to a placebo group. Two weeks into the study, although the LLLT group showed a higher reduction in ulcer size compared to the placebo group (47.5% vs. 29.4%), this difference did not reach statistical significance (p = 0.125). However, by four weeks into the study, significant differences emerged. The LLLT group exhibited significantly greater reductions in ulcer size compared to the placebo group at both two weeks (58% vs. 23.5%; p = 0.046) and four weeks (73.7% vs. 47.3%; p = 0.03) post-treatment initiation. Regarding complete healing of ulcers by the end of the follow-up period (20th week), 66.6% of ulcers in the LLLT group achieved complete healing compared to 38.4% in the placebo group. However, despite a shorter mean time to complete healing in the LLLT group (11 weeks; 95% CI, 7.3–14.7) compared to the placebo group (14 weeks; 95% CI, 8.76–19.2), this difference was not statistically significant (p = 0.470) .

2. in this study (MJ and EP, 2018) they used red laser 20 min daily for 15 days, In study group A, consisting of 50 subjects, there were 29 grade 2 ulcers and 21 grade 1 ulcers initially. By the end of 15 days, all grade 2 ulcers had either improved to grade 1 (96.6%) or completely healed (3.4%). Among the grade 1 ulcers, 7 (33.33%) remained in grade 1 while the majority, 14 (66.67%) ulcers, completely healed. In control group B, also comprising 50 subjects, there were 26 grade 2 ulcers and 24 grade 1 ulcers initially. At the end of 15 days, the majority of grade 2 ulcers (88.46%) remained in grade 2, while 3 ulcers (11.53%) improved to grade 1. All grade 1 ulcers remained unchanged, with none (0.00%) showing complete healing.

   These results suggest that LLLT using red light was more effective in promoting the healing of diabetic foot ulcers compared to the control group. Specifically, it led to a significant improvement in grade 2 ulcers, with the majority either improving to grade 1 or completely healing within the 15-day period.

3. In the study done by (Haze et al., 2022) they used in home B-cure laser device pulsed near infrared, application time 8 min area, In this study, patients with diabetic foot ulcers (DFUs) were divided into an active group receiving laser treatment and a sham group. Significant reductions in wound area were observed in the active group (median baseline vs endpoint: 10 $Cm^2$ vs 0.2 $Cm^2$, p = 0.002), but not in the sham group (median baseline vs endpoint: 7.9 $Cm^2$ vs 4.6 $Cm^2$, p = 0.63). Direct comparison of percent closure at the study termination revealed a significant healing effect of active laser treatment over sham

treatment (median % closure: active vs sham, 97% vs 49%). At the endpoint, a higher proportion of patients in the active group demonstrated over 90% closure compared to the sham group (7 out of 10 vs 1 out of 10, $p = 0.006$). Additionally, complete wound healing was achieved by more patients in the active group compared to the sham group (5 out of 10 vs 1 out of 10, $p = 0.051$).

4. In the study by (Darmaputri et al., 2020) that compared LLLT diode (group A 5 $J/Cm^{2)}$ and (group B 10 $J/Cm^2$), In Group A, there was a significant difference in the median size of DFUs between before therapy and at the end of week 4. While there was a reduction in wound size from week 1 to week 3 compared to before treatment, it was not statistically significant. The decrease in DFU size after 4 weeks of laser administration was 4.15 $mm^2$.

   In Group B, the wound size decreased significantly every week from the 1st to the 4th week. Complete wound closure was achieved in 5 subjects (35.7%) by the end of week 1, and by the end of week 3, 8 subjects (57.1%) had completed therapy due to adequate wound closure. The decrease in DFU size after 4 weeks of laser administration was 7.5 $mm^2$ .

   There was no significant difference in the decrease in DFU size between Group A and Group B after 4 weeks of therapy ($p = 0.178$). Additionally, there was no significant difference in the healing of DFUs using energy densities of 5 $J/Cm^2$ and 10 $J/Cm^2$. Both energy densities were deemed safe without any side effects, but using 5 $J/Cm^2$ is recommended to reduce the risk of side effects due to overdosing. However, the energy density of 10 $J/Cm^2$ be considered, especially for patients with hard-to-heal wounds or low socioeconomic status, as positive effects can be seen after just 1 week of therapy.

5. (Mousa et al., 2020) In this study, diode laser KTP 532 nm,was used , Empirical biophotomodulation therapy was performed circularly at the ulcer edge between the skin and ulcer bed, with a slow horizontal scanning manner to the bed, repeated 10 times. The movement speed was slow, delivering 2-3 pulses per area, and the total session time ranged from 5 to 10 minutes, adjusted according to ulcer size. This LLLT regimen was repeated three times for each patient, one week apart. After the first week of LLLT, all patients showed a dramatic response. Out of eleven patients, three (27.27%) were completely cured, while seven (63.36%) did not continue treatment despite significant improvement and formation of granulation tissue. Unfortunately, one patient (9.09%) ended up with toe amputation due to underlying osteomyelitis.

6. In the study done by (Esmael et al., 2023) usedSummus, platinum P4, class laser therapy Diode laser 4 wavelengths (980, 915, 810, and 650), The within-group comparison revealed a statistically significant reduction in WSA within both sequential and separate groups ($p < 0.05$), while there was no significant improvement in WSA in the control group after treatment compared to before-treatment values.

When comparing between groups, there was a statistically significant difference in mean values of WSA measured after treatment (p-value = 0.003). The sequential and separate groups showed lower values of WSA compared to the control group (p-values = 0.002 and 0.008, respectively). However, there was no significant difference between the sequential and separate groups (p-value = 0.361).

## 3.6 Granulation tissue formation

In a study by Minatel found that the ulcer granulation rate in the LLLT group was statistically greater than that in the control group (87.0 ± 4.96 vs. 30.8 ± 11.24, P = 0.0004) at the end of a 90-day treatment period (Minatel et al., 2009). Mathur et al. observed that the ulcers of the LLLT group had more granulation tissue than those of the control group (Mathur et al., 2017). In the study done by mousa increased fibrous tissue thickness and activated fibroblasts were observed, showing significant improvement post-LLLT (p=0.024 and p=0.038, respectively).(Mousa et al., 2020).

## 3.7 Treatment-related adverse events

None of the included studies reported that adverse events occurred during LLLT treatment

## Discussion

The current review assessed 11 studies that investigated the use of Low-Level Laser Therapy (LLLT) for the treatment of Diabetic Foot Ulcers (DFU). The included studies varied in their parameters, with wavelengths ranging from 532 to 980 nm, power density ranging from 4 to 400 mW, and fluence ranging from 0.8 to 10 $J/Cm^2$.

Upon evaluating and interpreting these 11 studies, it was determined that the most effective and commonly used type of laser for DFU treatment is the semiconductor diode laser. The studies that demonstrated a significant reduction in ulcer size utilized LLLT with a wavelength between 632 and 680 nm, power density between 4 and 10 $J/Cm^2$, and power output between 20 and 50 mW. These treatments were administered at least three times per week for a duration of one month.

Furthermore, the studies that reported notably high rates of complete healing were those in which LLLT was applied daily and for a longer period of time. These findings suggest that the application of LLLT with shorter wavelengths (632 to 685 nm), a power density of 50 $mW/Cm^2$, fluence between 4 and 10 $J/Cm^2$, an irradiation time of 30 to 80 seconds, and a distance of 1 cm from the ulcer for scanning application, as well as a distance of 1 cm between points for punctual application, at least three times a week for a month, can be beneficial for patients with DFU. However, longer treatment durations of approximately three months and a higher frequency of application may be necessary to achieve complete healing.

In four studies the reduction of ulcer area was significant compared with placebo but the patients couldn't achivie complete closure because of inadequate study duration less than 4 weeks , also in the study by kaviani the statisticaly significant result achivied after 4 weeks of therapy not 2 , this concludes that for higher healing rates and complete ulcer closure the duration should be at least three times per week for a month or more.

Minatel's study revealed that after 90 days, 58.3% of ulcers in group two had completely healed, and 75% had achieved 90-100% healing in the LLLT group. In contrast, only one ulcer in the control group, which received a placebo, had fully healed, and no other ulcers reached 90% healing. This suggests that a two-week timeframe is relatively short for assessing the effectiveness of LLLT in healing chronic ulcers. It is likely that the longer the duration of LLLT therapy, the higher the rate of ulcer closure. Consequently, further clinical trials should prioritize determining the optimal therapeutic duration for LLLT.(Minatel et al., 2009)

In the study by (El Rasheed et al., 2017), the effectiveness of Low-Level Laser Therapy (LLLT) was compared with Pulsed Electromagnetic Field (PEMF) therapy. Group A was subjected to 0.5 Gauss Pulsed Electromagnetic Field therapy, while Group B received 10 $J/cm^2$ Infra-red laser therapy at a wavelength of 904 nm. Both treatments were administered three times a week for a duration of 10 minutes over a period of four weeks.

Upon analysis, it was observed that both groups exhibited significant reductions in wound surface area and colony count after the treatment. There were no significant differences between the two groups in terms of pre-treatment measures. However, post-treatment, Group B, which received infra-red laser therapy, demonstrated a significantly greater reduction in wound surface area compared to Group A, favoring the use of infra-red laser therapy.

Although both therapies showed efficacy in reducing wound size and colony count, infra-red laser therapy exhibited superior outcomes in terms of wound healing. This suggests that infra-red laser therapy may be a more effective treatment option for wound healing compared to Pulsed Electromagnetic Field therapy.

One study by (Sandoval Ortíz et al., 2014) was conducted to compare the effects of Low-Level Laser Therapy (LLLT) and High Voltage Pulsed Current (HVPC) on wound healing, with a control group (CG) receiving standard wound care (SWC). The SWC included daily wound care activities such as irrigation, debridement, dressing changes, and patient education. The HVPC group received HVPC in addition to SWC, while the LLLT group received LLLT along with SWC.

The HVPC treatment involved 45-minute sessions three times a week, using specific parameters and electrode placement. On the other hand, LLLT consisted of punctual application of a semiconductor laser diode laser with a wavelength of 680 nm, power of 59mW, and energy density of 2 J/Cm$^2$, three times a week.

After 16 weeks, the healing rates were found to be similar across all groups. The closure of wounds was achieved in 77.7% of participants in the LLLT group, 80% in the HVPC group, and 66% in the CG. There were no significant differences observed between the groups in terms of healing proportions, nerve function, or quality of life. However, it is worth noting that at the 16th week, both LLLT and HVPC groups showed less dispersion and were closer to achieving 100% healing compared to the CG. Furthermore, the LLLT group demonstrated a significant improvement in mobility after 6 weeks of intervention.

(El-Kader et al., 2015) compared laser (4 J/Cm$^2$; 3 times weekly), ultrasound (3 MHz; 0.5 W/ Cm$^2$; 5 minutes; 1:5; 3 times weekly), and hyperbaric oxygen therapy (HBO; 90 minutes; 5 days weekly) for 2 months. The study involved 45 non-insulin dependent diabetic patients aged 35-50 years with grade II foot ulcers, divided into three equal groups: laser therapy (Group A), hyperbaric oxygen therapy (HBO, Group B), and pulsed ultrasound therapy (Group C), alongside standard medical treatment.

Laser therapy involved 20-minute sessions three times a week for two months, using a He-Ne scanning type laser at 4 J/Cm$^2$ intensity. Ultrasound therapy consisted of 5-minute sessions three times a week for two months, with a 3 MHz frequency and 0.5 W/Cm$^2$ intensity. HBO therapy entailed 90-minute sessions five days a week for eight weeks at 2.5 absolute temperature air.

Statistical analysis revealed no significant improvement in ulcer surface area and volume before treatment across the groups. However, after treatment, significant differences were observed in ulcer surface area and volume between the groups. HBO therapy showed the most significant improvement in accelerating ulcer healing compared to laser or ultrasound therapy alone.

In conclusion, adding hyperbaric oxygen therapy to standard medical treatment was found to be more effective in accelerating the healing of diabetic foot ulcers compared to laser or ultrasound therapy alone.

In the study by (Carvalho et al., 2016) involving 32 decompensated type II diabetic patients with ulcers, participants were randomly assigned to one of four groups: Control, Low-Level Laser Therapy (LLLT), Essential Fatty Acids (EFA), or LLLT associated with EFA (LEFA). LLLT involved specific parameters of laser application (wavelength of 658 nm, power of 30 mW, and an application time of 80 seconds (4 J/Cm$^2$)), while EFA group received calendula oil application daily. LEFA group underwent both LLLT and calendula oil application. Pain reduction was significant in both LLLT and LEFA groups, indicating an analgesic effect. Wound area reduction was significant in LEFA and LLLT groups, while the control group did not show significant changes. These findings suggest that LLLT, whether alone or in combination with calendula oil, effectively reduces pain and promotes wound healing in diabetic patients, potentially mitigating complications associated with Diabetes Mellitus.

Analysis of the included studies also showed benefits of LLLT in relieving pain. Among the studies that reported a significant pain reduction with LLLT compared with control, used similar parameters (658 nm; 30 mW; 4 J/Cm$^2$). Therefore, pain reduction can be considered a therapeutic effect of LLLT. (Samaneh et al., 2015)

Most patients suffering from DFUs complain about pain and insomnia, especially at night, which results in bad mood and poor quality of life. Positive effects of LLLT have been established in pain control for a variety of musculoskeletal conditions (Bjordal et al., 2006), also two RCTs reported the effectiveness of LLLT in improving pain. Feitosa et al. characterized pain from zero to ten, where ten was the maximum of pain and zero the absence of it. They found that in the LLLT group, the pain relief action was markedly reduced from an average of 9 to that of 5, with a significant improvement in mood and the capacity to independently move around and with a return to basic daily life activities, resulting in a better mood and quality of life, while no patient in the control group reported early relief from pain (Feitosa et al., 2015). Moreover, Minate reported that patients in the LLLT group began to report pain relief as early as 1 week of treatment, felt less pain and were able to sleep better at night; this improvement increased progressively with time (Minatel et al., 2009). These subjective findings were consistent with other reports that also indicated that phototherapy relieves pain and accelerates the resolution of inflammation (Gur et al., 2004).

(de Alencar Fonseca Santos et al., 2018) used the Visual Analog Scale (VAS) to assess pain intensity and found that there was no significant difference between the LLLT group and the control group in improving pain. Another study (Carvalho et al., 2016) used the Brief Pain Inventory Questionnaire and the VAS, and found that pain was significantly improved after LLLT. In other aspects of pain, studies showed that LLLT reduced the pain of eating, drinking, and toothbrushing for patients with recurrent aphthous stomatitis. (Albrektson et al., 2014), LLLT could also benefit pain in patients with osteoarthritis(Dima et al., 2017), However, another study showed that LLLT did not improve pain in postpartum women with a right mediolateral episiotomy after normal birth.(Alvarenga et al., 2017) Therefore, the effect of LLLT in improving pain may be related to the nature and source of the pain. Current studies show that the effect of LLLT in improving pain in patients with DFUs is not clear, and more research is needed.

Reliable scientific evidence is essential to guide the use of LLLT in clinical practice for management of DFU. The results of the included studies show that LLLT accelerate the healing of ulcers, promoting higher reducing size and higher complete healing rate relative to controls. In general, the participants of control groups received conventional treatment, which consisted mainly of dressings, pressure offloading, in addition to surgical debridement and antibiotic therapy if necessary. Our review found a significantly greater size reduction rate in the LLLT.

# Chapter four

## conclusion

In conclusion, the comprehensive review of 11 studies assessing Low-Level Laser Therapy (LLLT) for the treatment of Diabetic Foot Ulcers (DFU) reveals promising results. The semiconductor diode laser, particularly with wavelengths between 632 and 680 nm, power density of 4 to 10 $J/Cm^2$, and power output of 20 to 50 mW, emerges as the most effective modality for reducing ulcer size and promoting healing. Treatment frequencies of at least three times per week for a month, with longer durations and higher frequencies for optimal results, demonstrate notable benefits including pain relief, granulation tissue formation, and enhanced healing rates.

While significant reductions in ulcer size were observed in studies with shorter durations, complete closure often required longer treatment periods of at least one month or more. Notably, studies with inadequate durations failed to achieve complete closure, emphasizing the importance of prolonged therapy for optimal outcomes.

Importantly, the safety profile of LLLT for DFU appears favorable, with no reported adverse effects across the reviewed studies. However, to solidify the role of LLLT in DFU management, further research is warranted. Future studies should focus on larger sample sizes, extended follow-up periods, and precise parameter optimization to validate and enhance the efficacy of LLLT in treating DFUs.

In summary, while LLLT shows promise as a safe and effective modality for DFU treatment, continued investigation and refinement of treatment protocols are essential to fully unlock its therapeutic potential and integrate it into standard clinical practice.

# Refrences :


1. ALBREKTSON, M., HEDSTRÖM, L. & BERGH, H. 2014. Recurrent aphthous stomatitis and pain management with low-level laser therapy: a randomized controlled trial. *Oral surgery, oral medicine, oral pathology and oral radiology,* 117**,** 590-594.
2. ALVARENGA, M. B., DE OLIVEIRA, S. M. J. V., FRANCISCO, A. A., DA SILVA, F. M. B., SOUSA, M. & NOBRE, M. R. 2017. Effect of low-level laser therapy on pain and perineal healing after episiotomy: a triple-blind randomized controlled trial. *Lasers in surgery and medicine,* 49**,** 181-188.
3. BJORDAL, J. M., JOHNSON, M. I., IVERSEN, V., AIMBIRE, F. & LOPES-MARTINS, R. A. B. 2006. Low-level laser therapy in acute pain: a systematic review of possible mechanisms of action and clinical effects in randomized placebo-controlled trials. *Photomedicine and Laser Therapy,* 24**,** 158-168.
4. CARDOSO, V. S., DOS SANTOS, C. M., DA ROCHA, R. B. & HAZIME, F. A. 2021. Dose-response and efficacy of low-level laser therapy on diabetic foot ulcers healing: Protocol of a randomized controlled trial. *Contemporary Clinical Trials,* 110**,** 106561.
5. CARVALHO, A. F. M. D., FEITOSA, M. C. P., COELHO, N. P. M. D. F., REBÊLO, V. C. N., CASTRO, J. G. D., SOUSA, P. R. G. D., FEITOSA, V. C. & ARISAWA, E. A. L. S. 2016. Low-level laser therapy and Calendula officinalis in repairing diabetic foot ulcers. *Revista da Escola de Enfermagem da USP,* 50**,** 0628-0634.
6. CHUNG, H., DAI, T., SHARMA, S. K., HUANG, Y.-Y., CARROLL, J. D. & HAMBLIN, M. R. 2012. The nuts and bolts of low-level laser (light) therapy. *Annals of biomedical engineering,* 40**,** 516-533.
7. DARMAPUTRI, P., NUSDWINURINGTYA, N., MURDANA, N., TARIGAN, T. J. E. & FRISKA, D. 2020. Comparison Low-Level Laser Therapy (LLLT) of 10J/cm2 and 5 J/cm2 Energy Density for Healing Diabetes Foot Ulcer. *Journal Of The Indonesian Medical Association,* 70**,** 16-22.
8. DE ALENCAR FONSECA SANTOS, J., CAMPELO, M. B. D., DE OLIVEIRA, R. A., NICOLAU, R. A., REZENDE, V. E. A. & ARISAWA, E. Â. L. 2018. Effects of low-power light therapy on the tissue repair process of chronic wounds in diabetic feet. *Photomedicine and laser surgery,* 36**,** 298-304.
9. DIMA, R., FRANCIO, V. T., TOWERY, C. & DAVANI, S. 2017. Review of literature on low-level laser therapy benefits for nonpharmacological pain control in chronic pain and osteoarthritis. *trials,* 5**,** 6.
10. EL-KADER, A., MAHMOUD, S. & ASHMAWY, E. M. 2015. Impact of Different Therapeutic Modalities on Healing of Diabetic Foot Ulcers. *European Journal of General Medicine,* 12.
11. EL RASHEED, N. A. A., MAHMOUD, N. F., HAMADA, H. A. & EL KHATIB, A. 2017. Pulsed electromagnetic fields versus laser therapy on enhancing recovery of diabetic foot ulcer: A single blind randomized controlled trial. *Biomedical Research (0970-938X),* 28.
12. ESMAEL, H. F. A., EL BASIOUNY, M. S., MORSY, M. I., GAWAD, H. A. E. S. A., HSSEIN, A. A. & ABDALLAH, T. E. 2023. The Effect Of Combined 650 Nm And Infrared Laser On Chronic Diabetic Foot Ulcer Surface Area: A Randomized Controlled Trial. *Journal of Pharmaceutical Negative Results***,** 199-207.

13. FEITOSA, M. C. P., CARVALHO, A. F. M. D., FEITOSA, V. C., COELHO, I. M., OLIVEIRA, R. A. D. & ARISAWA, E. Â. L. 2015. Effects of the Low-Level Laser Therapy (LLLT) in the process of healing diabetic foot ulcers. *Acta cirurgica brasileira,* 30**,** 852-857.
14. GUR, A., SARAC, A. J., CEVIK, R., ALTINDAG, O. & SARAC, S. 2004. Efficacy of 904 nm gallium arsenide low level laser therapy in the management of chronic myofascial pain in the neck: A double-blind and randomize-controlled trial. *Lasers in surgery and medicine,* 35**,** 229-235.
15. HAZE, A., GAVISH, L., ELISHOOV, O., SHORKA, D., TSOHAR, T., GELLMAN, Y. N. & LIEBERGALL, M. 2022. Treatment of diabetic foot ulcers in a frail population with severe co-morbidities using at-home photobiomodulation laser therapy: a double-blind, randomized, sham-controlled pilot clinical study. *Lasers in Medical Science,* 37**,** 919-928.
16. HOURELD, N. & ABRAHAMSE, H. 2005. Low-level laser therapy for diabetic foot wound healing.(Wound care). *The Diabetic Foot,* 8**,** 182.
17. KAVIANI, A., DJAVID, G. E., ATAIE-FASHTAMI, L., FATEH, M., GHODSI, M., SALAMI, M., ZAND, N., KASHEF, N. & LARIJANI, B. 2011. A randomized clinical trial on the effect of low-level laser therapy on chronic diabetic foot wound healing: a preliminary report. *Photomedicine and laser surgery,* 29**,** 109-114.
18. KHARROUBI, A. T. & DARWISH, H. M. 2015. Diabetes mellitus: The epidemic of the century. *World journal of diabetes,* 6**,** 850.
19. KIM, J., NOMKHONDORJ, O., AN, C. Y., CHOI, Y. C. & CHO, J. 2023. Management of diabetic foot ulcers: a narrative review. *Journal of Yeungnam Medical Science,* 40**,** 335.
20. MATHUR, R., SAHU, K., SARAF, S., PATHEJA, P., KHAN, F. & GUPTA, P. 2017. Low-level laser therapy as an adjunct to conventional therapy in the treatment of diabetic foot ulcers. *Lasers in medical science,* 32**,** 275-282.
21. MINATEL, D. G., FRADE, M. A. C., FRANÇA, S. C. & ENWEMEKA, C. S. 2009. Phototherapy promotes healing of chronic diabetic leg ulcers that failed to respond to other therapies. *Lasers in Surgery and Medicine: The Official Journal of the American Society for Laser Medicine and Surgery,* 41**,** 433-441.
22. MJ, L. P. & EP, K. B. 2018. Effect of low level laser therapy on diabetic foot ulcers: a randomized control trial. *International Surgery Journal,* 5**,** 1008-1015.
23. MOSCA, R. C., ONG, A. A., ALBASHA, O., BASS, K. & ARANY, P. 2019. Photobiomodulation Therapy for Wound Care: A Potent, Noninvasive, Photoceutical Approach. *Advances in Skin & Wound Care,* 32**,** 157-167.
24. MOUSA, M. J., AL SAFFAR, H. S. & AL-HINDY, H. 2020. Low Level Laser (Biophotomodulation) Therapy for the Treatment of Diabetic Foot Ulcers with 532 nm KTP Laser Induces Wound Healing, Fibroblast Proliferation and Over-expression of TGF-β. *Systematic Reviews in Pharmacy,* 11**,** 396-403.
25. OYEBODE, O. A., JERE, S. W. & HOURELD, N. N. 2023. Current Therapeutic Modalities for the Management of Chronic Diabetic Wounds of the Foot. *Journal of Diabetes Research,* 2023.
26. RAJA, J. M., MATURANA, M. A., KAYALI, S., KHOUZAM, A. & EFEOVBOKHAN, N. 2023. Diabetic foot ulcer: A comprehensive review of pathophysiology and management modalities. *World J Clin Cases,* 11**,** 1684-1693.

27. SALVI, M., RIMINI, D., MOLINARI, F., BESTENTE, G. & BRUNO, A. 2017. Effect of low-level light therapy on diabetic foot ulcers: a near-infrared spectroscopy study. *Journal of Biomedical Optics,* 22**,** 038001-038001.
28. SAMANEH, R., ALI, Y., MOSTAFA, J., MAHMUD, N. A. & ZOHRE, R. 2015. Laser therapy for wound healing: A review of current techniques and mechanisms of action. *Biosci, Biotech Res Asia,* 12**,** 217-23.
29. SANDOVAL ORTÍZ, M. C., HERRERA VILLABONA, E., CAMARGO LEMOS, D. M. & CASTELLANOS, R. 2014. Effects of low level laser therapy and high voltage stimulation on diabetic wound healing. *Revista de la Universidad Industrial de Santander. Salud,* 46**,** 107-117.
30. TANTAWY, S. A., ABDELBASSET, W. K., KAMEL, D. M. & ALRAWAILI, S. M. 2018. A randomized controlled trial comparing helium-neon laser therapy and infrared laser therapy in patients with diabetic foot ulcer. *Lasers in medical science,* 33**,** 1901-1906.
31. TATA, D. B. & WAYNANT, R. W. 2011. Laser therapy: a review of its mechanism of action and potential medical applications. *Laser & Photonics Reviews,* 5**,** 1-12.
32. WANG, X., YUAN, C. X., XU, B. & YU, Z. 2022. Diabetic foot ulcers: Classification, risk factors and management. *World J Diabetes,* 13**,** 1049-1065.
33. YAMANY, A. A. & SAYED, H. M. 2012. Effect of low level laser therapy on neurovascular function of diabetic peripheral neuropathy. *Journal of Advanced Research,* 3**,** 21-28.