https://africanjournalofbiomedicalresearch.com/index.php/AJBR
Afr. J. Biomed. Res. Vol. 28(3s) (April 2025); 55-67
Review Article

Berberine Nanoparticles as a Promising Intervention for Diabetic-Wound Healing: A Comprehensive Review

Neetu Vishwakarma[1]*, Abhay Gupta[1], Ghanshyam Lodhi[1], Digvijay Kumar[1], Vaibhav Yadav[1], Deepti Jain[2], Ram Singh Bishnoi[2]

[1*,2] School of Pharmaceutical Sciences Rajiv Gandhi Proudyogiki Vishwavidyalaya, Bhopal, 462033 MP, India

***Corresponding author:** Neetu Vishwakarma
*Tel.: +91-9981861093 mail: vishwakarmaneetu.21@gmail.com

Abstract:
Diabetic-wounds is a very big burden in real time over the health care, as these-wounds take long time to heal and have high probability to get complicacies. Most traditional treatment methods do not provide very effective solutions to manage these wounds. Nevertheless, with the advent of nanotechnology, new alternatives for the design of different therapeutic approaches are now available. Berberine, an alkaloid found in several plant species, acclaimed for a variety of pharmacological applications, has also shown promising role in wound healing. Overview: the following comprehensive review seeks to provide an in-depth insight to the use of Berberine nanoparticles in the treatment of diabetic wounds. This review discusses the groundwork, formulation techniques, and relevant preclinical & clinical researches of Berberine nanoparticles that have shown improved efficiency in wound healing.
This introduction can be a read on diabetic wound pathophysiology and then moves on to the significant therapeutic windows required by new methods. This review seeks to explore the therapeutic potential of Berberine, through wound healing, yet this has not been fully translated for clinical application. Subsequently, the focus will be on nanoparticle-based Berberine delivery systems, detailing the advantages and the formulation strategies of Berberine nanoparticles. The path of Berberine in wound healing, which are anti-inflammatory, anti-oxidant, both pro and anti-angiogenic, and promote the growth of damaged tissue,zwill be discussed. In addition, we summarize preclinical studies demonstrating the efficiency of Berberine nanoparticles both in-vivo and in-vitro. Taken together, this review brings together the current information related to Berberine nanoparticle with diabetic wounding healing, providing a foundation for future work in this emerging field.

Keywords: Wound, Diabetic, Berberine, Nanoparticle & Healing.

***Author of correspondence: Email:** *vishwakarmaneetu.21@gmail.com*

Received - 1/04/2025 acceptance- 3/04/2025

DOI: https://doi.org/10.53555/AJBR.v28i3S.7370

1. Introduction

Diabetic-wounds present a major challenge in medical research, as they often exhibit delayed healing and increased susceptibility to infections.[1] In recent years, the development of innovative interventions using nanotechnology has shown promising potential in addressing this pressing issue.[2] One such intervention gaining attention is the utilization of berberine

nanoparticles.[3]Berberine, a natural compound found in various medicinal plants, possesses anti-inflammatory, antimicrobial, and wound healing properties.[4,5] By leveraging the unique characteristics of nanotechnology, re-searchers have-been able to improve the therapeutic effectiveness of berberine, resulting in improved healing outcomes for diabetic wounds.[6-10] In this comprehensive review, we delve into the advancements and potential of berberine nanoparticles as an exciting intervention for diabetic wound healing.[11] We explore the underlying mechanisms, research findings, and future prospects, shedding light on this cutting-edge approach towards revolutionizing diabetic wound management.

1.1 Diabetic wounds

Diabetes is a chronic disease where the body does not make enough insulin.[12] The body needs insulin to breakdown sugar due to excess amount of sugar is present in bloodstream and harm to body that causing a diabetic wound.[13] A diabetic wound is an ulcer or sore that occurs on the feet, heel or toes if patient is diabetic. High blood sugar can lead to nerve damage, impaired sweating, dry and cracked skin, toenail infections, damaged skin tissue that exposes the layers at depth and foot deformities that cause increasing the risk of a bacterial infection. [14,15,16]

1.2 Classification of diabetic wounds

One of the most commonly cited diabetic wound classification systems was first described by Meggitt in 1976, and popularized by Wagner in 1981.[6,7] The system is based mainly on wound depth and consists of six wound grades. These include grade a (intact skin), grade 1 (superficial ulcer), grade 2 (deep ulcer to tendon, bone, or joint), grade 3 (deep ulcer with abscess or osteomyelitis), grade 4 (forefoot gangrene), and grade 5 (whole-foot gangrene).[17]

1.2.1 The Grade-0 Wounds:

Grade -0 wounds, also known as "pre-ulcers" or "preliminary lesions," refer to the earliest stage of skin damage that can potentially develop into diabetic foot ulcers. These wounds are characterized by intact skin but may exhibit signs of inflammation, such as redness or warmth, and increased sensitivity.[18] Pre-ulcerative regions or past ulcer sites that have completely recovered with epithelial tissue following the excision of hyperkeratosis and nonviable tissue are classified as Grade-0 wounds. Since the callouses typically cover up frank ulcerations, a grade-0 wound can only be recognized after reducing any regional hyperkeratosis.[17]

1.2.2 The Grade 1 Wounds:

Grade 1 wounds, also known as "pre-stage 1 ulcers," are the earliest characteristic signs of skin damage that can potentially lead to diabetic foot ulcers. These wounds appear as an area of erythema, or reddening of the skin, and can be identified by their warmth and increased sensitivity.[22] Grade 1 wounds are usually painful to the touch, and the area may feel painful to walk on. The skin is not yet broken or blistered in grade 1 wounds, but early intervention is critical to prevent further progression.[23] Grade-I wound is superficial, involving partial or full thickness skin but not reaching tendon, capsule, or bone. If there are significant signs of purulence or fluctuance in the wound, additional investigation to uncover a more severe infection is necessary. [19,21]

1.2.3 The Grade II Wound:

Grade-2 wounds, also known as superficial ulcers, are a moderate stage of skin damage commonly observed in diabetic foot ulcers.[21] These wounds extend beyond the skin's surface, involving the dermis and potentially exposing underlying tissues. Grade 2 wounds typically exhibit partial thickness loss, with the ulcer appearing as a shallow or crater-like lesion. These ulcers may be accompanied by surrounding redness, swelling, and possible drainage.[18]The Grade-II wound indicates deeper involvement compared to Grade-I, affecting tendons or joint capsules but not extending to the bone.[19, 20]

1.2.4 The Grade- III Wound:

Grade-3 wounds, also known as full-thickness ulcers, are more severe and extensive than Grade 1 and Grade 2 wounds. These wounds penetrate the full thickness of the skin and affect underlying structures like muscles, tendons, or bones.[23] They often appear as deep craters or cavities with noticeable tissue loss and may emit a foul odor due to necrotic tissue or infection. Although not definitive, probing to bone strongly correlates with osteomyelitis. Three characteristics of a Grade-III wound are exposed bone, active infection, and vascular insufficiency. [20,21]

Classification by University of Texas: See Table 1

1.3 Pathophysiology of Diabetic Wound formation

The process of diabetic-wound development is intricate, involving various factors, both inherent and external, that can impact the skin's ability to heal.[24] Elevated blood sugar levels over prolonged periods, common in diabetes, can harm blood vessels and nerves, reducing blood flow and sensation in the feet and lower legs. Additionally, this illness encourages the skin's advanced glycation end products (AGEs) to accumulate, hindering normal cellular functions like collagen and elastin production.[25] This combination of neuropathy, reduced blood supply, and impaired wound healing heightens the risk of injury, particularly foot ulcers, in diabetic individuals. Moreover, structural changes such as calluses, bone deformities, and biomechanical imbalances due to neuropathy can increase pressure and friction on the skin, further exacerbating wound formation. [26]

Diabetic - ulcers typically, develop in three stages. Initially, neuropathy triggers the formation of a callus, with motor-neuropathy causing foot-deformities and sensory-neuropathy reducing sensation, thus increasing the risk of injury.[26] Moreover, autonomic neuropathy can induce skin dryness, exacerbating callus formation. Persistent trauma to the callus ultimately results in

subcutaneous bleeding, followed by erosion and ulceration. [24]

2. Phases of Diabetic Wound Healing.

2.1 Diabetic wound - healing:

Wound - healing is the innate mechanism by which the body repairs damaged tissue and reinstates its functionality. Following an injury, the body triggers a series of reactions to facilitate tissue integrity restoration.[25] These reactions unfold in four consecutive stages: hemostasis, inflammation, proliferation, and remodeling. Each stage serves distinct biological purposes and must unfold in the proper sequence, timing, and intensity for effective healing.[30] Disruption of any of these stages by various factors can result in insufficient or compromised tissue repair.[26]

2.2 Phases of diabetic wound healing:

The multifaceted nature is such that it interferes with each phase of wound healing as follows:

2.2.1. Inflammatory phases:

Wounds are classified as either open or closed, with open wounds encompassing lacerations, punctures, abrasions, incisions, and avulsions.[24] Upon injury, bleeding and vasoconstriction occur at the wound-site due to a release of chemical mediators like adrenaline, thromboxane, and prostaglandins.[21] This initial response leads to the aggregation of platelets and thrombin, forming a platelet plug. Subsequently, vasodilation ensues, allowing blood vessel contents to leak into the wound area, facilitating the infiltration of white blood cells and initiating the inflammatory phase.[26]

Within 24 hours, resident mast cells release mediators that attract neutrophils to eliminate debris, dead cells, and microbes. Additionally drawn to the area, macrophages release growth factors such platelet-derived growth-factor, endothelial-growth factors (EGF), & vascular-endothelial-growth-factor (VEGF) that are essential in preventing infection [PDGF], and interleukin-1 [IL-1]. The combined action of these factors contributes to the development of a chronic wound.[26]

2.2.2. Proliferative Phase:

The onset of the proliferative phase is marked by the gradual decrease in immune-cells & the migration of fibroblasts into wound - site. This phase is most intricate at the, molecular level, involving a multitude of cellular processes.[27] Different biochemical messengers such as [PDGF – BB], [TGF – β], and Fibronectin facilitate epithelial-mesenchymal transition.[30] Fibroblasts deposit collagen-matrix that supports epithelial-cell migration, activated by macrophages through (PDGF, TGF-β, EGF, and IGF-1). Subsequently, fibroblasts assume this role, resulting in wound contraction and eventual closure.[28] Meanwhile, neo - vascularization takes place to repair the damaged microvasculature, which involves the movement of endothelial cells and the formation of a new blood supply network in response to hypoxia and chemical signals. Ultimately, re-epithelialization happens through the migration of keratinocytes.[26]

2.2.3. Remodeling Phases:

This phase, often lasting a year or longer, involves continuous restructuring of the wound, with granulation of the tissue observed. During this period, collagen undergoes conversion from type III to type I, resulting in enhanced strength and increased crosslinking between existing and newly regenerated tissue fibers. However, the tissue strength does not fully return to its original state. [27,28]

3. Factors Contributing to Impaired Diabetic Wound Healing

Several factors can contribute to impair healing of diabetic wounds.[34] These factors can include:
See Table 2

4. Need for Advanced Therapeutic Approaches.

There is a need for advanced therapeutic approaches in the management of diabetes due to the complex nature of the disease and the challenges it presents.[31] Here are a few reasons why advanced therapies are necessary:
See Table 3

5. Berberine and Its Therapeutic Potential:

5.1 Overview of Berberine:

Berberine, a natural alkaloid found in various medicinal plants like goldenseal, Coptis, Oregon grape, barberry, and tree turmeric, possesses a range of pharmacological properties.[32] It has been utilized in Ayurvedic, Chinese, and other traditional medicinal systems for centuries, demonstrating proven medicinal efficacy.[32] Berberine has garnered significant attention as a therapeutic agent for conditions such as hyper-lipidemia, diabetes, metabolic- syndrome, polycystic-ovary syndrome, obesity, fatty-liver diseases & coronary-artery disease. Its wide array of pharmacological effects indicates its potential as a therapeutic agent for managing various clinical conditions. [33]

6.0 Types of Nanoparticles:

A. Nanosuspensions
B. Solid lipid Nanoparticles
C. Nanoshells
D. Nanopores
E. Nano film [31]

6.1 Methods of preparation of Nanoparticles:

As will be covered below, NPs are made from lipid, emulsifier, and water/solvent utilizing several techniques.

6.1.1. High-pressure uniformity
6.1.2. Convective blending
6.1.3. Homogenization by cold
6.1.4. High homogenization and ultrasonication
6.1.5. Ultrasonication of the probe
6.1.6. Ultrasonic bathing
6.1.7. Diffusion-Solvent Emulsification Method

6.1.8. Injecting a solvent
6.1.9. The method of solvent evaporation
6.1.10. Method based on micro-emulsion
6.1.11. Method of spray-drying
6.1.12. The double-emulsion approach
6.1.13. The method of precipitation
6.1.14. Dispersion of ultrasonography in films [35,36]

6.2 Advantages of Nanoparticles.

Nanoparticles offer several advantages due to their unique properties and size. Here are some of their key advantages:

6.2.1. Increased Surface Area: Nanoparticles exhibit a significant surface-to-volume ratio, allowing for efficient interactions with other materials. This property is particularly beneficial in applications such as catalysis, drug delivery, and sensing.[37]

6.2.2. Enhanced Reactivity: Due to their small size, nanoparticles often exhibit enhanced chemical reactivity and catalytic activity compared to bulk materials. This makes them useful in various chemical reactions and industrial processes.[38]

6.2.3. Improved Mechanical Properties:

Incorporating nanoparticles into materials can enhance their mechanical properties, such as strength, hardness, and flexibility. This is especially beneficial in the development of advanced materials for aerospace, automotive, and construction industries.[39.43]

6.2.4. Unique Optical and Electronic Properties: Nanoparticles can exhibit size-dependent optical and electronic properties, which can be manipulated for various applications. For example, quantum dots enable precise color tuning, while metallic nanoparticles can enhance the efficiency of solar cells and sensors.[38,39]

6.2.5. Targeted Drug Delivery: Nanoparticles have the capability to encapsulate drugs and transport them to precise locations within the body, enhancing their effectiveness in treatment while minimizing side effects. This targeted drug delivery approach shows potential for personalized medicine and addressing a range of diseases.[38]

6.2.6. Versatile Energy Applications: Nanoparticles are utilized in energy storage and conversion systems. Their large surface area and distinctive properties enable nanoparticles to improve the functionality of batteries, fuel cells, and solar cells.[37,39]

6.2.7. Environmental Advantages: Nanoparticles have the potential to improve environmental sustainability. They can be used to remediate contaminated soils and water, efficiently capture pollutants, and enable more efficient energy utilization.[39]

6.3 Features of medication delivery systems based on nanoparticles:

Drug delivery systems based on nanoparticles are novel approaches that use small particles at the nanoscale to deliver therapeutic medicines to targeted body locations.[40] These systems have many benefits over conventional medication delivery methods:

6.3.1. Enhanced drug-stability- Nano-particles can shield drugs from degradation, enzymatic breakdown, & premature release, improving their stability and prolonging their circulation time in the body.[41]

6.3.2. Controlled release- Nanoparticles can be engineered to release drugs in a controlled manner, allowing for either sustained or targeted drug delivery.[44] This regulated release mechanism can improve therapeutic efficacy, reduce side effects, and promote patient compliance.

6.3.3. Improved drug solubility and bioavailability- The poor solubility of many medications in biological fluids like water can hinder their absorption and effectiveness.[45] These weakly soluble medications can become more soluble and bioavailable with the help of nanoparticles, increasing their therapeutic efficacy.[46]

6.3.4. Targeted drug delivery- Nanoparticles can undergo surface modification with ligands &/or anti-bodies, enabling them to selectively identify and bind to target cells or tissues.[48] This targeting capability facilitates precise drug delivery to specific sites, reducing unintended effects and maximizing therapeutic outcomes.

6.3.5. Protection against drug clearance- Nanoparticles can shield drugs from clearance mechanisms in the body, such as enzymatic degradation or renal excretion, thereby extending their circulation time and increasing their concentration at the target site.[49]

6.3.6. Combination therapy- Drug-delivery systems based on nano-particles have the capacity to encapsulate multiple drugs or therapeutic agents, facilitating combination therapy. [46]This strategy can enhance treatment results through synergistic effects, simultaneous action on multiple targets, or sequential release of different drugs.[50]

6.3.7. Imaging and diagnostic applications- Nanoparticles can be tailored to transport imaging agents like fluorescent dyes or magnetic nanoparticles, allowing for the real-time observation and tracking of drug distribution within the body. This functionality is valuable for diagnostics and evaluating the efficacy of treatment.[48,49]

6.3.8. Imaging and diagnostic applications- Nanoparticles can be designed to transport imaging agents like fluorescent dyes or magnetic nanoparticles, facilitating the live visualization and tracking of drug dispersion within the body. This feature serves diagnostic purposes and evaluates treatment efficacy.[50]

7. Formulation Strategies for Berberine Nanoparticles

Formulating berberine nanoparticles involves selecting appropriate materials and techniques to effectively encapsulate berberine within Nano sized particles. Here are some common formulation strategies for berberine nanoparticles.[51,52]

7.1. Polymer-based nanoparticles: Polymers such as poly,(lactic-co-glycolic-acid) (PLGA), chitosan, or poly-ethylene-glycol (PEG) can be used to form nanoparticles through techniques like nanoprecipitation, emulsion, or solvent evaporation.[53] These polymers provide stability, control drug release, and protect berberine from degradation.

7.2. Lipid-based nanoparticles: Lipid-based nanoparticles like solid-lipid nano-particles (SLNs) or nano-structured lipid carriers (NLCs) can be utilized to encapsulate berberine. [54]These systems offer higher drug-loading capacities and improved stability. Lipid-based nanoparticles can be prepared via high-pressure homogenization or micro emulsion techniques.

7.3. Self-assembled nanoparticles: Self-assembled nanoparticles can be formed by utilizing the amphiphilic properties of certain materials.[54] For example, phospholipids or surfactants can self-assemble into micelles or liposomes that encapsulate berberine. Self-assembled nanoparticles offer improved solubility and enhanced drug delivery.[55]

7.4. Nano emulsions: Nano emulsions are another approach to formulate berberine nanoparticles. By dispersing berberine in an oil phase with the help of surfactants and co-surfactants, followed by emulsification, Nano-sized droplets of berberine can be obtained.[56] This technique provides improved drug solubility and stability.

7.5. Co-precipitation method: In this method, berberine can be encapsulated in nanoparticles along with other materials, like natural or synthetic polymers, through a co-precipitation process.[56,57] This approach allows for the modification of drug release profiles and provides stability to the nanoparticles.

7.6. Electrostatic interactions: Berberine can be loaded into nanoparticles through electrostatic interactions with oppositely charged polymers.[58] For example, berberine can be complexed with positively charged polymers like chitosan or polyethyleneimine to form nanoparticles.

7.7. Surface modification: Surface modification of the nanoparticles with ligands or targeting moieties can be employed to enhance specific interactions with target cells / tissues.[52] This modification facilitates targeted-delivery and controlled release of berberine.[58]

It's essential to consider that choosing the right formulation strategy depends on factors like desired particle size, drug loading efficiency, stability, and intended use.[58] Each strategy has its pros and cons, and optimization is necessary to attain the desired characteristics of berberine nano-particles. Additionally, formulation strategies need validation through equally in-vitro and in-vivo studies to confirm their efficacy & safety. [57]

8. Characterization Techniques for Berberine Nanoparticles

Characterizing berberine nanoparticles is essential to understanding their physicochemical properties, stability, drug release behavior, and potential in therapeutic applications.[59-64] Here are some common characterization techniques used for berberine nanoparticles:

See Table 4

These characterization techniques provide valuable information on the physiochemical properties, stability, drug-release behavior, and interactions of berberine nano-particles.[65-69] Combining multiple techniques can offer comprehensive insights into the formulation and potential applications of these nanoparticles.

9. Preclinical studies for Berberine loaded Nanoparticles formulation

Preclinical studies are an essential part of the drug-development-process, including the evaluation of berberine nanoparticles.[70,71] These studies aim to assess the safety, efficacy, pharmacokinetics, and pharmacodynamics of berberine nanoparticles in animal models before moving on to clinical trials.

Here is an overview of the preclinical study process: See Table 5

10. In vitro Evaluation of Berberine Nanoparticles

The in vitro evaluation of berberine nanoparticles involves conducting various experiments and assays to assess their physicochemical properties, cytotoxicity, cellular uptake, and therapeutic efficacy. [72] Here are some key aspects of the in vitro evaluation process:

10.1. Physicochemical Characterization: Physical & chemical properties of berberine nano-particles are analyzed using techniques such as dynamic light scattering (DLS) or particle size analysis to determine their size, size distribution, and stability.[73] Surface charge or zeta potential measurements can provide insights into the nanoparticle surface properties.

10.2. Drug Release Studies: Studies on the in vitro release of drugs are conducted to assess the berberine release profile from nanoparticles. These studies help determine the release kinetics, sustained release behavior, and the influence of various factors like pH, temperature, or formulation parameters on drug release.[76]

10.3. Cytotoxicity Assessment: Berberine nanoparticle cytotoxicity is evaluated using cell viability assays such as MTT, XTT, or resazurin-based assays.[74] Cells are exposed to different concentrations of berberine nanoparticles, and their effects on cell viability and proliferation are determined. Control groups without nanoparticles or with a free berberine solution are typically included for comparison.[72]

10.4. Cellular Uptake Studies: Fluorescently labeled or radiolabeled berberine nanoparticles can be used to evaluate their cellular uptake. Cells are incubated with the nanoparticles, and techniques such as confocal microscopy, flow cytometry, or fluorescence spectroscopy are employed to visualize and quantify nanoparticle internalization into cells.[76]

10.5. Intracellular Localization: To determine the intracellular distribution of berberine nanoparticles, techniques such as confocal microscopy or fluorescence imaging can be used. [75] Specific organelle markers can help identify whether the nanoparticles accumulate in particular cellular compartments, such as the cytoplasm or nucleus.

10.6. Mechanism of Action Studies: Berberine nanoparticles' mechanism of action can be investigated through various assays related to their intended therapeutic targets.[77] For example, in cancer research, cell cycle analysis, apoptosis assays, or Western blotting can be conducted to examine the impact of berberine

nanoparticles on cancer cell proliferation, cell cycle progression or apoptotic pathways.[72]

10.7. Cellular Signaling Pathways: Berberine nanoparticles may have an impact on cellular signaling pathways. Through gene expression profiling, Western blotting, or enzyme activity assays, their effects on specific cellular pathways such as MAPK, PI3K/Akt, or NF-κB can be evaluated.[76]

10.8. Cell-Cell Interaction Studies: Co-culture or cell migration assays can be conducted to examine how berberine nanoparticles influence cellular interactions, proliferation, or migration in the context of specific diseases or tissue models.[74]

10.9. Stability and Compatibility Studies: Berberine nanoparticles may undergo stability and compatibility assessments using techniques such as (FTIR), (DSC), or spectroscopic methods to ensure the constancy and reliability of the nano-particles during storage. [77]

11. Berberine Nanoparticles' In-vivo Effectiveness in Animal Models

To evaluate the in- vivo ability of berberine nano-particles, animal models are utilized to assess their therapeutic effects. Here is an overview of the process:

11.1. Animal Model Selection: Appropriate animal models are chosen based on the specific disease or condition targeted by berberine nanoparticles. Commonly used animals include mice, rats, rabbits, or larger species like dogs or primates. The choice of animal-model depends on factors including disease similarity, physiological relevance, and availability.[78]

11.2. Disease Induction: If necessary, animals are induced with the specific disease or condition being studied. This can involve various methods such as chemical induction, genetic modification, transplantation, or exposure to pathogens.[78]

11.3. Treatment Administration: Animals are treated with berberine nanoparticles via the desired route of administration, such as oral gavage, intravenous injection, intraperitoneal injection, or topical application. Different dosage regimens may be used, including single or multiple doses or sustained-release formulations.[79]

11.4. Monitoring Parameters: Relevant parameters are measured to evaluate the therapeutic efficacy of berberine nanoparticles. This can include monitoring changes in disease symptoms, tumor growth, biochemical markers, blood parameters, organ function, or structural changes in tissues through imaging techniques such as MRI, CT scans, or histopathological analysis.

11.5. Therapeutic Outcome Evaluation: The therapeutic outcomes of berberine nanoparticles are assessed by quantifying their effects on relevant disease-specific endpoints. For example, tumor size reduction, inhibition of disease progression, improvement in organ function, increased survival rate, or alleviation of disease symptoms are evaluated. [79]

11.6. Control Groups: Control groups are essential to compare the effects of berberine nanoparticles. Negative control groups receive a placebo or vehicle, while positive control groups may receive a standard treatment or comparative intervention. These control groups help establish the efficacy and specificity of berberine nanoparticles in the studied animal model. [79]

11.7. Data Analysis: The relevance of berberine nanoparticles' therapeutic effects is ascertained by statistically analyzing data from animal tests. Data may be presented through graphs, tables, or statistical values to support the conclusions drawn from the study.[80]

11.8. Ethical Considerations: Animal studies must adhere to ethical guidelines and regulations, including obtaining proper approvals from ethics committees, minimizing animal suffering, and ensuring humane treatment throughout the experimental procedure. [80]

By conducting in vivo efficacy studies, researchers gain valuable insights into the therapeutic potential of berberine nanoparticles, allowing for further optimization, dose determination, and translation to human clinical trials. These animal studies bridge the gap between preclinical and clinical stages and provide essential evidence for the safety and effectiveness of berberine nanoparticles in treating specific diseases or conditions.

12. Future Prospects

Despite the promising findings, several avenues for further research and development of berberine nanoparticles for diabetic wound healing remain open. Here are a few future prospects to consider:

12.1. Optimization of Formulation- Continued efforts should focus on refining the formulation parameters of berberine nanoparticles to enhance their stability, release kinetics, and targeted delivery to the wound site. Novel nanoparticle designs and surface modifications could also be explored to improve their interaction with wound tissues.

12.2. Mechanistic Understanding- Further investigations are needed to elucidate the underlying mechanisms by which berberine nanoparticles exert their effects on diabetic wound healing. This will help in better understanding the molecular pathways involved and identifying potential targets for intervention.

12.3. Safety and Toxicity Assessment- Comprehensive studies evaluating the long-term safety and potential toxicity of berberine nanoparticles should be carried out to ensure their safe clinical translation. Bioavailability, bio distribution, and clearance studies are also crucial to assess the pharmacokinetics of berberine nanoparticles in vivo.

12.4. Clinical Trials- Clinical-trials are warranted to determine the efficacy, safety, and optimal dosage of berberine nano-particles in human subjects with diabetic wounds. Well-designed, randomized controlled trials will provide valuable evidence for their clinical utility

12.5. Combination Therapies- Exploring the synergistic effects of berberine nanoparticles with other wound healing agents or growth factors may further enhance their therapeutic potential. Combination therapies could potentially address multiple-aspects of the complex wound-healing process in diabetic individuals.

13. Conclusion

In summary, this comprehensive review highlights the promising potential of berberine nanoparticles as an intervention for diabetic wound healing. The studies discussed demonstrate that berberine nanoparticles exhibit enhanced wound healing properties compared to free berberine. The unique physic-chemical properties of nano-particles, such as their controlled release, improved bioavailability, and targeted delivery, contribute to their enhanced efficacy in promoting wound healing in diabetic individuals. Berberine nanoparticles have shown positive effects on various stages of wound healing, including inflammation modulation, angiogenesis promotion, extracellular matrix synthesis, and antimicrobial activity.

These nanoparticles have been found to accelerate wound closure, reduce wound size, and improve the quality of healed tissue in experimental models of diabetic wounds. Furthermore, berberine nanoparticles exhibit additional benefits such as anti-oxidant activity, anti-inflammatory effects, and regulation of glucose metabolism, which are of particular importance in the context of diabetic wound healing. In conclusion, berberine nanoparticles hold great promise as an intervention for diabetic wound healing. Continued research and development efforts, along with clinical trials, will pave the way for their translation into clinical practice, providing a much-needed solution for improving diabetes-related wound healing outcomes

Acknowledgement

I would like to express my deepest sense of appreciation and profound gratitude to my reserved HOD Dr. Deepti Jain, School of Pharmaceutical Sciences, RGPV, Bhopal for her guidance, constant encouragement, valuable suggestions and kindest helps without which I would never have succeeded to complete this study.I shall extremely thankful and pay my gratitude to my guide Dr. Ram Singh Bishnoi, Assistant Professor, School of Pharmaceutical Sciences, RGPV Bhopal for his excellent guidance, encouragement, valuable suggestions and kindest help.

Conflicts of interest

The authors report no conflicts of interest.

References

1. Feng, P., Luo, Y., Ke, C., Qiu, H., Wang, W., Zhu, Y & Wu, S. (2021). Chitosan-based functional materials for skin wound repair: Mechanisms and applications. Frontiers in Bioengineering and Biotechnology, 9, 650598.
2. Kesharwani P, Gorain B, Low SY, et al. Nanotechnology based approaches for anti-diabetic drugs delivery. *Diabetes Res Clin Pract*. 2018;**136**:52–77. doi: 10.1016/j.diabres.2017.11.018
3. Dixon D, Edmonds M. Managing diabetic foot ulcers: pharmacotherapy for wound healing. *Drugs*. 2021;**81**(1):29–56. doi: 10.1007/s40265-020-01415-8
4. Patel, S., Srivastava, S., Singh, M. R., & Singh, D. (2019). Mechanistic insight into diabetic wounds: Pathogenesis, molecular targets and treatment strategies to pace wound healing. Biomedicine & Pharmacotherapy, 112, 108615.
5. Blanco-Fernandez B, Castano O, Mateos-Timoneda MA, Engel E, Perez-Amodio S. Nanotechnology approaches in chronic wound healing. *Adv Wound Care*. 2021;**10**(5):234–256. doi: 10.1089/wound.2019.1094
6. Haque ST, Saha SK, Haque ME, Biswas N. Nanotechnology-based therapeutic applications: in vitro and in vivo clinical studies for diabetic wound healing. *Biomater Sci*. 2021;**9**(23):7705–7747. doi: 10.1039/d1bm01211h.
7. Stark WJ, Stoessel PR, Wohlleben W, Hafner A. Industrial applications of nanoparticles. *Chem Soc Rev*. 2015;**44**(16):5793–5805. doi: 10.1039/C4CS00362D.
8. Zhang Y, Luo J, Zhang Q, Deng T. Growth factors, as biological macromolecules in bioactivity enhancing of electrospun wound dressings for diabetic wound healing: a review. *Int J Biol Macromol*. 2021;**193**(Pt A):205–218. doi: 10.1016/j.ijbiomac.2021.09.210
9. Berger AG, Chou JJ, Hammond PT. Approaches to modulate the chronic wound environment using localized nucleic acid delivery. *Adv Wound Care*. 2021;**10**(9):503–528. doi: 10.1089/wound.2020.1167
10. Madhukiran D, Jha A, Kumar M, Ajmal G, Bonde GV, Mishra B. Electrospun nanofiber-based drug delivery platform: advances in diabetic foot ulcer management. *Expert Opin Drug Deliv*. 2021;**18**(1):25–42. doi: 10.1080/17425247.2021.1823966
11. Alavi M, Rai M. Topical delivery of growth factors and metal/metal oxide nanoparticles to infected wounds by polymeric nanoparticles: an overview. *Expert Rev Anti Infect Ther*. 2020;**18**(10):1021–1032. doi: 10.1080/14787210.2020.1782740
12. Choudhury H, Pandey M, Lim YQ, et al. Silver nanoparticles: advanced and promising technology in diabetic wound therapy. *Mater Sci Eng C Mater Biol Appl*. 2020;**112**:110925. doi: 10.1016/j.msec.2020.110925
13. Mirza RE, Koh TJ. Contributions of cell subsets to cytokine production during normal and impaired wound healing. *Cytokine*. 2015;**71**(2):409–412. doi: 10.1016/j.cyto.2014.09.005
14. Han G, Ceilley R. Chronic wound healing: a review of current management and treatments. *Adv Ther*. 2017;**34**(3):599–610. doi: 10.1007/s12325-017-0478-y
15. Wang Y, Wu Y, Long L, et al. Inflammation-responsive drug-loaded hydrogels with sequential hemostasis, antibacterial, and anti-inflammatory behavior for chronically infected diabetic wound treatment. *ACS Appl Mater Interfaces*. 2021; **13**(28):33584–33599. doi: 10.1021/acsami.1c09889

16. Bai Q, Han K, Dong K, et al. Potential applications of nanomaterials and technology for diabetic wound healing. *Int J Nanomedicine*. 2020;**15**:9717–9743. doi: 10.2147/IJN.S276001.
17. Lavery, L. A., Armstrong, D. G., & Harkless, L. B. (1996). Classification of diabetic foot wounds. The Journal of Foot and Ankle Surgery, 35(6), 528-531.
18. Uccioli, L., Faglia, E ., Mont icone , G., Favale s, F., Durola , L.,Aldeghi, A., Quarantiello, A., Calia, P., Menzinger, G. Manufactured shoes in the prevention of diabetic foot ulcers. Di abetesCare 18:1376-1378, 1995.
19. Grayson, M. L., Balaugh, K., Levin, E., Karchmer, A. W. Probing to bone in infected pedal ulcers. A clinical sign of underlying osteomyelitis in diabetic patients. J. Am. Med Assoc. 273:721-723,1995.
20. Caputo, G. M. Infection: investigation and management, ch. 18. In The Foot in Diabetes, 2nd ed , pp. 203-211, edited by A. J. M.Boulton, H. Connor, and P. R. Cavanagh, Wiley and Sons,Chichester, 1994.
21. Giacalone, V. F., Krych, S. M., Harkless, L. B. The University of Texas Health Science Center at San Antonio: experience with foot surgery in diabetics. J. Foot Ankle Surg. 33:590-597, 1994.
22. Apelqvist, J., Ragnarson-Tennval, G., Persson, U ., Larsson, J.Diabetic foot ulcers in a multidisciplinary setting-an economic analysis of primary healing and healing with amputation. J . Intern.Med . 235:463-471, 1994.
23. Gibbons, G. W., Marcaccio, E. J., Jr ., Burgess, A. M., Pomposelli,F. B., Jr., Freeman , D. V., Campbell, D. R , Miller, A. , LoGerfo ,F. W. Improved quality of diabetic foot care, 1984 vs. 1990:reduced length of stay and cost s, insufficient reimbursement. Arch.Surg. 128:576 -581 , 1993.
24. Mirza RE, Koh TJ. Contributions of cell subsets to cytokine production during normal and impaired wound healing. *Cytokine*. 2015;**71**(2):409–412. doi: 10.1016/j.cyto.2014.09.005
25. Han G, Ceilley R. Chronic wound healing: a review of current management and treatments. *Adv Ther*. 2017;**34**(3):599–610. doi: 10.1007/s12325-017-0478-y
26. Armstrong DG, Boulton AJM, Bus SA. Diabetic Foot Ulcers and Their Recurrence. N Engl J Med. 2017 Jun 15;376(24):2367-2375.
27. Falanga, V. (2005). Wound healing and its impairment in the diabetic foot. The Lancet, 366(9498), 1736-1743.
28. Mazayen, Z. M., Ghoneim, A. M., Elbatanony, R. S., Basalious, E. B., & Bendas, E. R. (2022). Pharmaceutical nanotechnology: from the bench to the market. Future Journal of Pharmaceutical Sciences, 8(1), 1-11.
29. Wang Y, Wu Y, Long L, et al. Inflammation-responsive drug-loaded hydrogels with sequential hemostasis, antibacterial, and anti-inflammatory behavior for chronically infected diabetic wound treatment. *ACS Appl Mater Interfaces*. 2021;**13**(28):33584–33599. doi: 10.1021/acsami.1c09889
30. Satish Patel, Shikha Srivastava, Manju Rawat Singh, Deependra Singh, Mechanistic insight into diabetic wounds: Pathogenesis, molecular targets and treatment strategies to pace wound healing, Biomedicine & Pharmacotherapy, Volume 112, 2019, 108615, ISSN 0753-3322.
31. . Bai Q, Han K, Dong K, et al. Potential applications of nanomaterials and technology for diabetic wound healing. *Int J Nanomedicine*. 2020;**15**:9717–9743. doi: 10.2147/IJN.S276001
32. Prajwala, B., Raghu, N., Gopenath, T. S., Shanmukhappa, B., Karthikeyan, M., Ashok, G., ... & Kanthesh, M. B. (2020). Berberine and its pharmacology potential: a review. Eur J Biomed, 7, 115-23.
33. Kumar, A., Chopra, K., Mukherjee, M., Pottabathini, R., & Dhull, D. K. (2015). Current knowledge and pharmacological profile of berberine: an update. European journal of pharmacology, 761, 288-297.
34. . Lai HJ, Kuan CH, Wu HC, et al. Tailored design of electrospun composite nanofibers with staged release of multiple angiogenic growth factors for chronic wound healing. *Acta Biomater*. 2014;**10**(10):4156–4166. doi: 10.1016/j.actbio.2014.05.001
35. Younis, F. A., Saleh, S. R., El-Rahman, S. S. A., Newairy, A. S. A., El-Demellawy, M. A., & Ghareeb, D. A. (2022). Preparation, physicochemical characterization, and bioactivity evaluation of berberine-entrapped albumin nanoparticles. Scientific Reports, 12(1), 1-16.
36. Zhang P, He L, Zhang J, et al. Preparation of novel berberine nano-colloids for improving wound healing of diabetic rats by acting Sirt1/NF-κB pathway. *Colloids Surf B Biointerfaces*. 2020;**187**:110647. doi: 10.1016/j.colsurfb.2019.110647.
37. Meng B, Li J, Cao H. Antioxidant and antiinflammatory activities of curcumin on diabetes mellitus and its complications. *Curr Pharm Des*. 2013;**19**(11):2101–2113.
38. Xia G, Zhai D, Sun Y, et al. Preparation of a novel asymmetric wettable chitosan-based sponge and its role in promoting chronic wound healing . *Carbohydr Polym*. 2020;**227**:115296. doi: 10.1016/j.carbpol.2019.115296.
39. Bairagi U, Mittal P, Singh J, Mishra B. Preparation, characterization, and in vivo evaluation of nano formulations of ferulic acid in diabetic wound healing. *Drug Dev Ind Pharm*. 2018;**44**(11):1783–1796. doi: 10.1080/03639045.2018.1496448
40. Kaymakcalan OE, Abadeer A, Goldufsky JW, et al. Topical α-gal nanoparticles accelerate diabetic wound healing. *Exp Dermatol*. 2020;**29**(4):404–413. doi: 10.1111/exd.14084.
41. Jing-jing Zhang, Rui Zhou, Li-juan Deng, Guang-zhao Cao, Yi Zhang, He Xu, Jing-yi Hou, Shang Ju, Hong-jun Yang, Huangbai liniment and berberine promoted wound healing in high-fat diet/ Streptozotocin-induced diabetic rats, Biomedicine & Pharmacotherapy, Volume 150, 2022, 112948, ISSN 0753-3322.

42. Bhagwat, R. R., & Vaidhya, I. S. (2013). Novel drug delivery systems: an overview. International Journal of Pharmaceutical sciences and research, 4(3), 970.
43. Shinde, N. C., Keskar, N. J., & Argade, P. D. (2012). Nanoparticles: Advances in drug delivery systems. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 3(1), 922- 929.
44. Patra, J. K., Das, G., Fraceto, L. F., Campos, E. V. R., Rodriguez-Torres, M. D. P., Acosta- Torres, L. S., ... & Shin, H. S. (2018). Nano based drug delivery systems: recent developments and future prospects. Journal of nanobiotechnology, 16(1), 1-33
45. Alsaad, A. A., Hussien, A. A., & Gareeb, M. M. (2020). Solid lipid nanoparticles (SLN) as a novel drug delivery system: A theoretical review. Syst Rev Pharm, 11, 259-73.
46. Sarangi, M. K., & Padhi, S. (2016). Solid lipid nano particles–a review. Drugs, 5, 718- Lingayat, V. J., Zarekar, N. S., & Shendge, R. S. (2017). Solid lipid nanoparticles: a review. Nanoscience and Nanotechnology Research, 4(2), 67-72.
47. Parhi, R., & Suresh, P. (2012). Preparation and characterization of solid lipid nanoparticles-a review. Current drug discovery technologies, 9(1), 2-16.
48. Afreen Sultana, Mina Zare, Vinoy Thomas, T.S. Sampath Kumar, Seeram Ramakrishna, Nano-based drug delivery systems: Conventional drug delivery routes, recent developments and future prospects, Medicine in Drug Discovery, Volume 15, 2022, 100134, ISSN 2590-0986,
49. Ms Priya Sharma, Dr Amarjeet Singh, & Dr Navjot Singh Sethi. (2024). Design and development of optimized berberine chloride formulation for treatment of diabetes. Journal of Population Therapeutics and Clinical Pharmacology, 31(3), 1642–1686.
50. Novel nano-insulin formulation modulates cytokine secretion and remodeling to accelerate diabetic wound healing. *Nanomedicine*. 2019;**15**(1):47–57. doi: 10.1016/j.nano.2018.08.013.
51. Gibbons WG. Lower extremity bypass in patients with diabetic foot ulcers. Surg Clin N Am 2003;83:659-669.
52. Kesharwani P, Gorain B, Low SY, et al. Nanotechnology based approaches for anti-diabetic drugs delivery. *Diabetes Res Clin Pract*. 2018;136:52–77. doi:10.1016/j.diabres.2017.11.018.
53. Kobyliak N, Abenavoli L, Kononenko L, Kyriienko D, Spivak M. Neuropathic diabetic foot ulcers treated with cerium dioxide nanoparticles: a case report. *Diabetes Metab Syndr*. 2019;**13**(1):228–234. doi: 10.1016/j.dsx.2018.08.027
54. Niemiec SM, Louiselle AE, Hilton SA, et al. Nanosilk increases the strength of diabetic skin and delivers CNP-miR146a to improve wound healing. *Front Immunol*. 2020;**11**:590285. doi: 10.3389/fimmu.2020.590285 .
55. Sener G, Hilton SA, Osmond MJ, et al. Injectable, self-healable zwitterionic cryogels with sustained microRNA - cerium oxide nanoparticle release promote accelerated wound healing. *Acta Biomater*. 2020;**101**:262–272. doi: 10.1016/j.actbio.2019. 11.014
56. Ren X, Han Y, Wang J, et al. An aligned porous electrospun fibrous membrane with controlled drug delivery - An efficient strategy to accelerate diabetic wound healing with improved angiogenesis. *Acta Biomater*. 2018;**70**:140–153. doi: 10.1016/j.actbio .2018.02.010
57. Gan J, Liu C, Li H, et al. Accelerated wound healing in diabetes by reprogramming the macrophages with particle-induced clustering of the mannose receptors. Biomaterials. 2019;219:119340. doi: 10.1016/j.biomaterials.2019.119340.
58. Feng Y, Chen L, Luo Q, Wu M, Chen Y, Shi X. Involvement of microRNA-146a in diabetic peripheral neuropathy through the regulation of inflammation. Drug Des Devel Ther. 2018;12:171–177. doi: 10.2147/DDDT.S157109.
59. Birdsall TC, Kelly GS. Berberine therapeutic potential of an alkaloid found in several medicinal plants. Altern Med Rev. 1997;2(2):94–103.
60. Bakshi J, Mehra M, Grewal S, Dhingra D, Kumari S. Synthesis, characterization, and evaluation of in vitro antimicrobial and anti-diabetic activity of berberine encapsulated in guar-acacia gum nanocomplexes. J Bioact Compat Polym. 2022;37(4):233–251.
61. Birdsall TC, Kelly GS. Berberine therapeutic potential of an alkaloid found in several medicinal plants. Altern Med Rev. 1997;2(2):94–103.
62. Park K-S, Kang K-C, Kim J-H, Adams DJ, Johng T-N, Paik Y-K. Differential inhibitory effects of protoberberines on sterol and chitin biosyntheses in Candida albicans. J Antimicrob Chemother. 1999;43(5):667–674.
63. Müller RH, Junghanns J. Drug nanocrystals/ nanosuspensions for the delivery of poorly soluble drugs. In: Torchilin VP, editor. Nanoparticulates as Drug Carriers. London: Imperial College Press; 2006. p. 307–328.
64. Zhang SL, Chang JJ, Damu GL, et al. Novel berberine triazoles: synthesis, antimicrobial evaluation and competitive interactions with metal ions to human serum albumin. Bioorg Med Chem Lett. 2013;23(4):1008–1012.
65. Patel RP, Patel MM. Solid-state characterization and dissolution properties of lovastatin hydroxypropyl-β-cyclodextrin inclusion complex. Pharm Technol. 2007;31(2):72–81.
66. Kakran M, Sahoo N, Li L, et al. Fabrication of drug nanoparticles by evaporative precipitation of nanosuspension. Int J Pharm. 2010;383(1):285–292.
67. Shid RL, Dhole SN, Kulkarni N, Shid SL. Formulation and evaluation of nanosuspension delivery system for simvastatin. Int J Pharm Sci Nanotechnol. 2014;7:2459–2476.
68. Jiang T, Han N, Zhao B, Xie Y, Wang S. Enhanced dissolution rate and oral bioavailability of simvastatin nanocrystal prepared by

sonoprecipitation. Drug Dev Ind Pharm. 2012;38 (10):1230–1239.
69. Leuner C, Dressman J. Improving drug solubility for oral delivery using solid dispersions. Eur J Pharm Biopharm. 2000;50(1):47–60.
70. Hancock BC, Parks M. What is the true solubility advantage for amorphous pharmaceuticals? Pharm Res. 2000;17(4):397–404.
71. Shid RL, Dhole SN, Kulkarni N, Shid SL. Formulation and evaluation of nanosuspension delivery system for simvastatin. Int J Pharm Sci Nanotechnol. 2014;7:2459–2476.
72. Pivodová V, Franková J, Galandáková A, Ulrichová J. In vitro AuNPs' cytotoxicity and their effect on wound healing. Nanobiomedicine. 2015;2:7. doi: 10.5772/61132.
73. Choudhury D, Jala A, Murty US, et al. In vitro and in vivo evaluations of berberine-loaded microparticles filled in-house 3D printed hollow capsular device for improved oral bioavailability. AAPS PharmSciTech. 2022;23:89. doi: 10.1208/s12249-022-02114-w.
74. Krishnan N, Velramar B, Ramatchandirin B, et al. Effect of biogenic silver nanocubes on matrix metalloproteinases 2 and 9 expressions in hyperglycemic skin injury and its impact in early wound healing in streptozotocin-induced diabetic mice. Mater Sci Eng C Mater Biol Appl. 2018;91:146–152. doi: 10.1016/j.msec.2018.05.020.
75. Dhilip Kumar SS, Houreld NN, Abrahamse H. Selective laser efficiency of green-synthesized silver nanoparticles by Aloe arborescens and its wound healing activities in normal wounded and diabetic wounded fibroblast cells: in vitro studies. Int J Nanomedicine. 2020;15:6855–6870. doi: 10.2147/IJN.S257204.
76. de Oliveira DR, Tintino SR, Braga MF, et al. In vitro antimicrobial and modulatory activity of the natural products silymarin and silibinin. Biomed Res Int. 2015;2015:292797.
77. Quan H, Cao Y-Y, Xu Z, et al. Potent in vitro synergism of fluconazole and berberine chloride against clinical isolates of Candida albicans resistant to fluconazole. Antimicrob Agents Chemother. 2006;50(3):1096–1099.
78. Ribeiro MC, Correa VLR, Silva F, et al. Wound healing treatment using insulin within polymeric nanoparticles in the diabetes animal model. Eur J Pharm Sci. 2020;150:105330.
79. Li F, Shi Y, Liang J, Zhao L. Curcumin-loaded chitosan nanoparticles promote diabetic wound healing via attenuating inflammation in a diabetic rat model. J Biomater Appl. 2019;34(4):476–486.
80. Samadian H, Zamiri S, Ehterami A, et al. Electrospun cellulose acetate/gelatin nanofibrous wound dressing containing berberine for diabetic foot ulcer healing: in vitro and in vivo studies. Sci Rep. 2020;10:831. doi: 10.1038/s41598-020-75894-2.

Figure and Table legends

Figures

Fig 1- Classification of Diabetic Wounds

Fig 2 -Structure of Berberine

Figures:

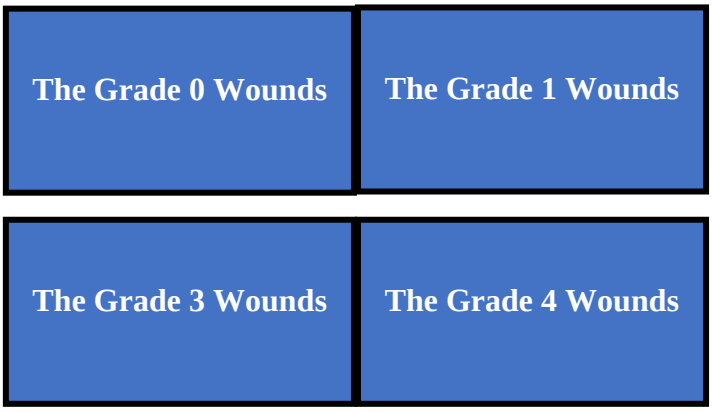

Fig 1- Classification of Diabetic Wounds

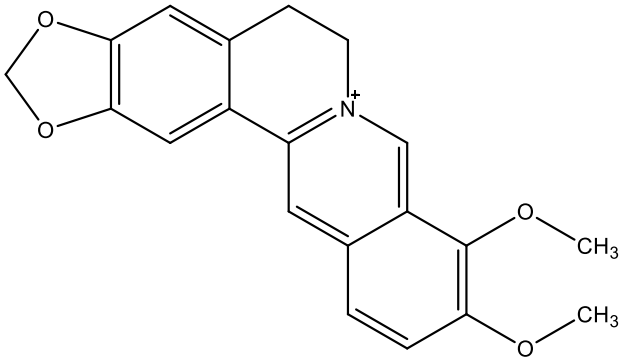

Fig 2 -Structure of Berberine

Tables:

Table 1- Wounds Classification by University of Texas

A	**Hyperglycemia**	Prolonged periods of high-blood sugar-levels can impairs normal wound healing process by affecting cellular function, prominent to delayed and ineffective healing.
B	**Neuropathy**	Diabetic neuropathy, or nerve damage, can effect damage of sensation in the feet and lower-extremities. This can result in unnoticed injuries, increased pressure on certain areas, and delayed wound healing.
C	**Peripheral vascular disease**	Diabetes can lead to the narrowing and hardening of blood vessels, known as peripheral-vascular-disease. Diminished blood-flow to wound location can restrict the supply of oxygen, nutrients, and immune-cells essential for successful healing.
D	**Impaired-immune function**	Diabetes can compromise the immune-system, increasing susceptibility to infections. Infection at the wound site can delay healing and lead to further complications.
E	**Chronic inflammation**	Diabetes can lead to a state of chronic, low-grade inflammation that can interfere with the normal wound healing process. Persistent inflammation can disrupt the development of new blood-vessels & the migration of cells necessary for tissue repair.
F	**Reduced collagen synthesis**	Diabetes can hinder the production and placement of collagen, an essential element of extracellular matrix crucial for tissue resilience and wound healing.
G	**Comorbidities**	Diabetes patients frequently also suffer from obesity, cardiovascular disease, and kidney disease, all of which can impede the healing of wounds.
H	**Medications**	Some medications frequently prescribed for diabetes management, like corticosteroids, may hinder wound healing because of their anti - inflammatory effects. [7]

Table-2 Factors Contributing to Impaired Diabetic Wound Healing

	GRADE - 0	**GRADE - 1**	**GRADE - 2**	**GRADE - 3**
STAGE-I	A Pre / post ulcerative lesion i,e fully epi-thelialized.	A surface-level wound that does not affect the tendon, capsule, or bone.	A-wound that reaches the tendon or joint, capsule.	A -wound that reaches the bone or joint.
STAGE-II	A lesion before or after ulceration that is fully covered with epithelial tissue but has an infection.	A surface-level wound without any impact on the tendon, capsule, or bone, yet accompanied by an infection.	A-wound that extends to the tendon or joint, capsule and is accompanied by an infection.	A -wound that penetrates to the bone or joint and has an infection
STAGE-III	A pre or post-ulcerative lesion that is fully epithelialized but has ischemia.	A superficial wound with ischemia that does not involve the tendon, capsule, or bone.	A- wound that reaches the tendon or joint, capsule and exhibits ischemia.	A- wound that extends to the bone or joint and exhibits ischemia.
STAGE-IV	A pre or post ulcerative lesion that is fully epithelialized but exhibits both infection / ischemia.	A surface-level wound without any impact on the tendon, capsule, or bone, but presenting both infection / / ischemia.	A-wound that extends to the tendon or joint, capsule and presents with both infection / / ischemia.	a wound that travels to the joint or bone and manifests as both an ischemia and an infection.

Table 3- Elements of Advance Therapeutic approaches

A	**Increasing prevalence and burden**	Diabetes is a global health epidemic, with increasing prevalence and associated healthcare costs. Advanced therapeutic approaches can help address the growing burden of the disease by improving outcomes and reducing complications.
B	**Prevention and management of complications**	Diabetes can result in a range of complications, including, diabetic neuropathy, retinopathy, nephropathy, and cardiovascular problems. Advanced therapies aim to not only prevent these complications but also manage them more effectively, resulting in enhanced quality of life for people with diabetes.
C	**Personalized-medicine**	Every person with diabetes is unique, and their response to medications and treatments can vary. Advanced therapeutic approaches can utilize personalized medicine strategies, such as genetic profiling, biomarker analysis, and tailored treatment plans, to optimize outcomes and minimize adverse effects.
D	**Enhanced wound healing**	Diabetic wounds, as mentioned earlier, can be challenging to heal. Advanced therapies, including growth-factors, bio-engineered skin-substitutes, hyperbaric-oxygen therapy, and advanced dressings, can promote more rapid and effective wound healing in diabetic patients
E	**Simplified management**	Diabetes management often requires multiple medications, frequent monitoring, and lifestyle modifications. Advanced therapies aim to simplify the management process by developing more convenient drug delivery systems, automated insulin administration devices, and digital health technologies that enhance self-monitoring and self-management
F	**Research and innovation**	Advanced therapeutic approaches drive research and innovation in the field of diabetes management. New discoveries and technologies help uncover novel treatment targets, develop more effective interventions, and advance our understanding of the disease

Table 4- Characterization Techniques for Berberine Nanoparticles

1	**Particle size and size distribution analysis**	Dynamic-light-scattering (DLS) or laser-diffraction-techniques can be utilized to assess the average size and poly-dispersity index (PDI) of berberine nano-particles. These methods provide information about the particle-size- distribution & stability of the nano-particles.
2	**Surface morphology analysis**	Transmission-electron-microscopy (TEM) and scanning - electron microscopy (SEM) are useful methods for analyzing the structure and surface appearance of berberine nanoparticles. These techniques offer details regarding the integrity, form, and surface characteristics of the nanoparticles.
3	**Zeta potential measurement**	Zeta potential analysis helps determine the surface charge of berberine nanoparticles. This information assists in understanding the stability, colloidal behavior, and potential interactions with biological components.
4	**Drug en-capsulation efficiency and loading-capacity**	The amount of berberine present in the nano-particles can be evaluated using techniques such as high-performance liquid-chromatography (HPLC) or ultraviolet-visible-spectroscopy (UV-Vis). These methods measure the drug en-capsulation effectiveness (%E-E) and drug-loading capacity (%D-L) of the nano-particles.
5	***In-vitro*-drug release- studies**	Dissolution or release studies can be conducted to assess the release pattern of berberine from nan-oparticles. These studies aid in comprehending the kinetics, duration, and mechanisms governing the drug- release from the nano-particles.
6	**Stability assessment**	Stability studies are crucial to assess the long-term stability of berberine nanoparticles. Parameters including, particle-size, zeta-potential, drug- content, and physical-integrity can be monitored over time under various storage conditions (e.g., temperature, pH, and light exposure).
7	**Thermal analysis:**	Methods like differential-scanning-calorimetry (DSC) or thermo-gravimetric analysis (TGA) are employed to examine the thermal characteristics, stability, and crystalline structure of berberine nano-particles. These analyses offer understanding of the physical state of the drug within the nano-particles.
8	**Fourier-transform infrared-spectroscopy (FTIR)**	FTIR-spectroscopy can be used to identify and analyze functional groups, molecular interactions, and potential chemical alterations in berberine nano-particles. This technique aids in evaluating the compatibility between the drug and the nano-particle formulation components.
9	**X-ray-diffraction (X-R-D):**	XRD-analysis offers insights into crystalline-structure of berberine and any changes induced by nano-particle formulation. XRD can help ascertain the physical state of the drug within the nano-particles.
10	**In vitro cytotoxicity and cellular uptake studies:**	Cell-based assays, such as MTT or cell viability assays, can be conducted to determine the cytotoxicity and cellular uptake behavior of berberine nanoparticles. These studies evaluate the biocompatibility and potential therapeutic efficacy of the nanoparticles. [23]

Table 5- Overview of the preclinical study process for Berberine loaded Nanoparticles

1	**Study Design**	Preclinical studies are designed based on established guidelines and regulations, taking into account factors such as the intended use of berberine nano-particles, target species, dosing regimen, and endpoints to be evaluated.
2	**Acute toxicity studies**	These studies involve exposing animals to various doses of berberine nano-particles to assess any immediate adverse effects or toxic reactions. Commonly used animals for acute toxicity studies include rodents (e.g., mice or rats). The maximum tolerated dose (MTD) is determined to guide further studies.
3	**Repeat-dose toxicity studies**	: In these studies, animals are dosed with berberine nano-particles repeatedly over an extended period to evaluate the potential toxic effects associated with long-term exposure. Different doses are tested, and various organs and tissues are examined for potential adverse effects.
4	**Pharmacokinetic studies**	These studies determine the ADME of berberine nano-particles in animal models. Blood and tissue samples are collected at various time points after administration to measure the concentration-time profiles and calculate pharmacokinetic parameters
5	**Bio distribution studies:**	Bio distribution studies assess the distribution of berberine nanoparticles within the body after administration. Techniques such as radiolabeling, fluorescence imaging, or mass spectrometry can be employed to track the nanoparticles and determine their accumulation in various organs and tissues
6	**Efficacy studies**	These studies evaluate the therapeutic effectiveness of berberine nano-particles in animal disease models. The nano-particles are administered, and relevant endpoints are measured to assess the desired therapeutic effects. For instance, in cancer models, tumor growth inhibition or regression may be evaluated.
7	**Safety pharmacology studies**	Safety pharmacology studies evaluate the potential impact of berberine nano-particles on vital organ functions, including the cardio-vascular, respiratory, and central-nervous-systems. These studies aim to identify any adverse effects that may result from administering the nano-particles.
8	**Genotoxicity and mutagenicity studies:**	These studies examine the potential genotoxic and mutagenic properties of berberine nanoparticles using *in-vitro* and *in-vivo* assays. Many techniques, for example the Ames test or chromosome aberration assays, may be employed.
9	**Immunotoxicity studies**	Immunotoxicity studies evaluate the potential impact of berberine nanoparticles on the immune system. These studies assess immune cell function, cytokine levels, and antibody responses to determine any immunomodulatory effects
10	**ADME studies**	Additional in-depth studies might be conducted to further investigate the ADME properties of berberine nanoparticles, including metabolism, elimination pathways, and potential drug-drug interactions.