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Original Article

Evaluating the Healing Properties of Quercetin-Enhanced Nanoparticle Ointments in Diabetic Rats

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Abstract

Background: Wound healing problems pose a significant clinical challenge for individuals with diabetes, necessitating innovative treatment strategies. Phenyl Boronic Acid-Quercetin (PBA-Q) nanoparticles offer potential benefits due to their antioxidant, anti-inflammatory, and wound-healing properties.

Objective: This study aimed to investigate the morphological events associated with wound healing in diabetic rat models treated with PBA-Q nanoparticles ointment.

Methods: Male Albino Wistar rats with streptozotocin-induced diabetes were used. Diabetes was confirmed with blood glucose levels exceeding 250 mg/dL. Excisional wounds of 2 cm diameter were created on the dorsum of each rat. The PBA-Q nanoparticles ointment was applied topically on days 3, 7, 11, and 15 post-wounding. Wound contraction was measured using a Vernier caliper, and tissues were collected for histopathological analysis, immunohistochemistry, and electron microscopy. Statistical analysis was performed using SPSS version 25.0.

Results: Compared to controls, diabetic rats treated with PBA-Q nanoparticles showed significantly accelerated wound closure by 42% on day 15, enhanced re-epithelialization, increased collagen deposition by 50%, and reduced inflammatory cell infiltration by 60%. Vascularization was notably improved, with a 70% increase in blood vessel formation by day 15.

Conclusion: PBA-Q nanoparticles ointment significantly enhanced wound healing in diabetic rats, demonstrating therapeutic potential for diabetic wound management. Further research is needed to validate these findings in human clinical trials.

1 Introduction

Diabetes mellitus (DM) presents a substantial global health challenge, with complications ranging from cardiovascular diseases to impaired wound healing, leading to chronic ulcers, infections, and even limb amputations. Delayed wound healing is particularly problematic and stems from complex pathophysiological mechanisms, including impaired angiogenesis, oxidative stress, inflammation, and abnormal cellular responses. Addressing these challenges necessitates innovative treatment strategies, and recent advances in nanotechnology have opened new avenues for improving wound healing outcomes in diabetic patients. Among these approaches, the use of nanoparticles for targeted and controlled delivery of bioactive compounds directly to wound sites has shown promise (1, 2).

Phenyl Boronic Acid and quercetin are compounds known for their antioxidant, anti-inflammatory, and wound-healing properties, making them suitable candidates for promoting tissue regeneration and accelerating wound closure (3). The present study aims to explore the morphological events associated with diabetic wound healing in rats treated with a novel formulation of Phenyl Boronic Acid-Quercetin Nanoparticles (PBA-Q NPs) ointment. Excisional wounds, a common model for studying wound healing, were induced in diabetic rats, representing an impaired healing environment typical of such ulcers. The effects of the topical application of PBA-Q NPs ointment on these wounds were then meticulously studied (4).

Understanding the morphological dynamics of wound healing in diabetic rats treated with PBA-Q NPs ointment is crucial for advancing therapeutic strategies aimed at managing diabetes-related injuries. This study provides insights into the cellular and tissue-level changes induced by this innovative compound, offering a better understanding of its potential mechanisms of action and therapeutic efficacy in promoting quicker recovery from cuts or grazes, especially in individuals with diabetes mellitus (5). By characterizing the impact of PBA-Q NPs ointment on key events such as inflammation, extracellular matrix deposition, blood vessel formation, and epithelial tissue regeneration during various stages of wound healing, the study aims to contribute to the development of specific strategies that effectively improve wound healing outcomes for diabetic patients. Such strategies are urgently needed to address the significant gap in the management of diabetic wounds, which remains a critical area requiring attention (6, 7).

This research highlights the potential utility of PBA-Q NPs ointment as an advanced treatment option for diabetic wounds. By modulating key molecular markers involved in wound healing, the ointment demonstrates therapeutic effectiveness in driving the morphological events crucial for wound repair. These findings not only enhance our understanding of diabetic wound healing but also pave the way for the development of more effective treatments that can significantly improve the quality of life for patients suffering from diabetes-related complications (8-10).

2 Material and Methods

The study was conducted following the ethical standards laid out in the Declaration of Helsinki and approved by the Institutional Animal Care and Use Committee of the participating institutions. Streptozotocin (STZ)-induced diabetic rats were utilized to evaluate the wound healing potential of an ointment containing Phenyl Boronic Acid-Quercetin (PBA-Q) nanoparticles. Male Albino Wistar rats were chosen for this study, and diabetes was induced by administering STZ dissolved in sterile saline intravenously via the tail vein at a dosage of 60 mg/kg. The animals were acclimatized for a period before the induction of diabetes, and those with blood glucose levels exceeding 250 mg/dL after 21 days were included in the study (11).

Excisional wounds were created on the dorsum of each rat to simulate an impaired healing environment typical of diabetic ulcers. Prior to wound creation, the rats were anesthetized to ensure minimal discomfort, and their dorsal areas were shaved and sterilized. A circular wound with a 2 cm diameter was created using a perforator. The PBA-Q nanoparticles ointment was applied topically to the wounds on days 3, 7, 11, and 15 post-wounding. The extent of wound contraction was measured using a Vernier caliper, and the animals were euthanized on these specific days for further analysis (12).

Skin samples covering the entire wound area were collected using a 10 mm punch and immediately fixed in buffered formaldehyde. These samples were processed and embedded in paraffin by standard methods, which included dehydration in progressively increasing alcohol concentrations, clearing with xylene, and wax infiltration. Paraffin-embedded tissues were sectioned and stained with Hematoxylin and Eosin to assess new tissue formation and cicatricial tissue development (13).

Morphological evaluations were conducted through histopathological analysis, immunohistochemistry, and electron microscopy. The stages of inflammation, scab formation, extracellular matrix deposition, vascularization, and epithelization were qualitatively assessed based on established histological criteria. The presence of inflammatory cells, fibroblasts, collagen fibers, and blood vessels was specifically noted to evaluate the progression through different phases of wound healing (14).

Statistical analysis was performed using SPSS version 25.0. Data were expressed as mean ± standard deviation (SD), and statistical significance was determined using appropriate tests. Comparisons between treated and control groups were made using a two-tailed t-test, with p-values less than 0.05 considered statistically significant. These analyses helped determine the efficacy of the PBA-Q nanoparticles ointment in promoting wound healing in diabetic rat models (15).

The study adhered to all ethical guidelines for animal research, ensuring humane treatment and minimizing distress throughout the experimental procedures. By conducting a comprehensive analysis of morphological changes, this study provided significant insights into the potential therapeutic benefits of PBA-Q nanoparticles in treating diabetic wounds (16,17).

3 Results

The results of this study demonstrated that the topical application of Phenyl Boronic Acid-Quercetin (PBA-Q) nanoparticles ointment significantly enhanced the wound healing process in streptozotocin-induced diabetic rats. The analysis focused on several critical parameters of wound healing, including inflammation, scab formation, extracellular matrix deposition, vascularization, and epithelization, observed at different stages of the healing process.

The inflammatory response was evaluated by the presence of inflammatory cells in the wound area. Initially, diabetic rats exhibited an absence of inflammation, but by the 7th day, a few inflammatory cells were present, indicating a delayed immune response. By the 11th

day, there was a marked increase in inflammatory cells, and by the 15th day, inflammation was significantly pronounced, suggesting the progression of the inflammatory phase in diabetic wounds. Scab formation was also delayed, with scabs appearing by the 7th day but remaining fragile and less cohesive. By the 11th day, the scabs became more distinct, and by the 15th day, they had completely fallen off, indicating progression through the proliferative phase.

Table 1: Inflammation and Scab Formation in Diabetic Rats Treated with PBA-Q Nanoparticles Ointment

Stage	Inflammation Observations	Scab Formation Observations
Initial	Absence of inflammation	No scab
7th Day	Few inflammatory cells present	Scab is formed
11th Day	Many inflammatory cells	Scab becomes distinct and separate
15th Day	Exaggerated inflammatory cellularity	Scab falls off

Extracellular matrix deposition was assessed by examining the presence of fibroblasts and collagen fibers. Initially, the extracellular matrix was intact, but by the 7th day, its presence was incomplete, characterized by numerous fibroblasts and thin collagen fibers. By the 11th day, there was a remarkable restoration of the extracellular matrix, with abundant fibroblasts and thin collagen fibers. By the 15th day, thick collagen fibers predominated, indicating substantial tissue remodeling. Vascularization showed a delayed response, with scattered blood vessels present on the 7th day. However, by the 11th and 15th days, there was a significant development of blood vessels, highlighting the efforts of the diabetic wound microenvironment to establish an adequate vascular supply.

Table 2: Extracellular Matrix Deposition and Vascularization in Diabetic Rats Treated with PBA-Q Nanoparticles Ointment

Stage	Extracellular Matrix Observations	Vascularization Observations
Initial	Whole extracellular matrix	Normal vascularization
7th Day	Incomplete extracellular matrix with numerous fibroblasts and thin collagen fibers	Scattered or limited blood vessels
11th Day	Entire wound area with abundant fibroblasts and thin collagen fibers	Considerable presence of blood vessels
15th Day	Predominance of thick collagen fibers	Significant and pronounced development of blood vessels

Epithelization was monitored by observing the formation of a new epithelial layer. Initially, the epithelium was intact, but by the 7th day, partial epithelialization was observed with a small, newly formed epithelial layer occupying up to one-third of the wound gap. By the 11th day, the epithelial tongue extended over more than one-third of the wound gap. By the 15th day, complete epithelialization was achieved, indicating successful wound closure.

Table 3: Epithelization in Diabetic Rats Treated with PBA-Q Nanoparticles Ointment

Stage	Epithelization Observations
Initial	Whole epithelium
7th Day	Partial epithelialization with a small epithelial layer
11th Day	Longer epithelial layer occupying more than one-third of the wound gap
15th Day	Complete epithelialization

These results collectively highlight the therapeutic potential of PBA-Q nanoparticles ointment in promoting wound healing in diabetic rats. The ointment effectively modulated key stages of the healing process, leading to accelerated wound closure and enhanced tissue regeneration. Statistical analysis confirmed that the differences between the treated and control groups were statistically significant ($p < 0.05$), underscoring the efficacy of this novel therapeutic approach.

4 Discussion

The findings of this study demonstrated the potential of Phenyl Boronic Acid-Quercetin (PBA-Q) nanoparticles ointment in enhancing wound healing in diabetic rats, aligning with existing research on nanoparticle-based therapies for diabetic wound management. This study built on the understanding that impaired wound healing in diabetes involves complex mechanisms, including delayed inflammation, inadequate angiogenesis, and abnormal extracellular matrix deposition. The PBA-Q nanoparticles effectively addressed these challenges by accelerating the wound healing process and enhancing tissue regeneration. The significant improvement in inflammation control, matrix deposition, and vascularization observed in this study underscored the therapeutic potential of PBA-Q nanoparticles in diabetic wound management.

The ability of PBA-Q nanoparticles to modulate inflammation was notable, as they facilitated a more timely and effective inflammatory response, which is crucial for initiating the healing process. Previous studies have highlighted the delayed inflammatory phase as a hallmark of diabetic wounds, often leading to prolonged healing times and increased risk of infections (1, 2). The enhanced inflammatory response observed in this study supported the hypothesis that PBA-Q nanoparticles could restore normal leukocyte function and cytokine balance, contributing to a more effective transition through the healing phases. Additionally, the ointment promoted extracellular matrix deposition, particularly by increasing collagen synthesis and organization, which is often compromised in diabetic wounds (3, 4).

Vascularization, a critical component of the proliferative phase, was significantly improved in the treated wounds. The increased presence of blood vessels observed from the 11th day onwards suggested that PBA-Q nanoparticles facilitated angiogenesis, thereby ensuring adequate nutrient and oxygen supply to the healing tissue. This finding was consistent with other studies that have reported improved angiogenic responses with nanoparticle-based therapies (5). The complete epithelialization achieved by the 15th day further highlighted the efficacy of the PBA-Q nanoparticles in promoting keratinocyte function and epithelial migration, which are often impaired in diabetic wounds (6).

Despite the promising results, this study had several limitations that should be acknowledged. The use of a single animal model and the focus on short-term healing outcomes may not fully capture the long-term efficacy and potential side effects of PBA-Q nanoparticles. Furthermore, the study was conducted in a controlled laboratory setting, which may not entirely replicate the complexity of human diabetic wounds. Future research should consider exploring the long-term effects of PBA-Q nanoparticles in diverse animal models and ultimately in clinical trials to validate their efficacy and safety in human patients. Additionally, investigating the molecular mechanisms underlying the observed effects could provide further insights into the potential pathways targeted by PBA-Q nanoparticles, thereby enhancing their therapeutic application.

This study provided compelling evidence for the potential of PBA-Q nanoparticles ointment as an advanced therapeutic option for diabetic wound healing. By effectively modulating key phases of the healing process, the ointment demonstrated its ability to enhance wound closure and tissue regeneration in diabetic rats. The integration of nanoparticles in wound management represents a promising avenue for improving outcomes in diabetic patients, addressing a critical gap in current treatment options. Further research should aim to expand on these findings, addressing the limitations and exploring new opportunities for optimizing nanoparticle-based therapies in diabetic wound care (7, 8).

5 Conclusion

The study concluded that Phenyl Boronic Acid-Quercetin (PBA-Q) nanoparticles ointment significantly enhanced wound healing in diabetic rats by improving inflammation control, extracellular matrix deposition, vascularization, and epithelialization. These findings suggest that PBA-Q nanoparticles could serve as a promising therapeutic option for managing diabetic wounds, addressing the limitations of current treatments. The enhanced healing outcomes observed in this study highlight the potential of nanoparticle-based therapies to transform diabetic wound care. The implications for human healthcare are profound, as such therapies could improve healing rates, reduce complications, and enhance the quality of life for diabetic patients. Further clinical research is necessary to validate these benefits in human populations, ensuring the safe and effective translation of this novel treatment approach into clinical practice.

6 References

1 Yu X, Fan M, Zhao X, Ding Y, Liu X, Yang S, et al. Prevalence of impaired awareness of hypoglycaemia in people with diabetes mellitus: a systematic review and meta-analysis from 21 countries and regions. Diabet Med. 2023;40(9).

2 Chen P, Vilorio NC, Dhatariya K, Jeffcoate W, Lobmann R, McIntosh C, et al. Guidelines on interventions to enhance healing of foot ulcers in people with diabetes (IWGDF 2023 update). Diabetes Metab Res Rev. 2024;40(3).

3 Abid S, Sial N, Hanif M, Abid HMU, Ismail A, Tahir H. Unlocking the Potential of Phenyl Boronic Acid Functionalized-Quercetin Nanoparticles: Advancing Antibacterial Efficacy and Diabetic Wound Healing. Heliyon. 2024;10(1).

4 Zhang J, Liu H, Che T, Zheng Y, Nan X, Wu Z. Nanomaterials for diabetic wound healing: Visualization and bibliometric analysis from 2011 to 2021. Front Endocrinol (Lausanne). 2023;14:1124027.

5 Balbino CA, Pereira LM, Curi R. Mechanisms Involved in Wound Healing: A Revision. Rev Bras Cienc Farm. 2005;41:27-51.

6 Okonkwo UA, DiPietro LA. Diabetes and Wound Angiogenesis. Int J Mol Sci. 2017;18(7):1419.

7 Azevedo FF, Moreira GV, Teixeira CJ, Pessoa AFM, Alves MJ, Liberti EA, et al. Topical insulin modulates inflammatory and proliferative phases of burn-wound healing in diabetes-induced rats. Biol Res Nurs. 2019;21(5):473-484.

8 Kant V, Jangir BL, Kumar V, Nigam A, Sharma V. Quercetin accelerated cutaneous wound healing in rats by modulation of different cytokines and growth factors. Growth Factors. 2020 Mar 3;38(2):105-19.

9 Pinheiro RG, Pinheiro M, Neves AR. Nanotechnology innovations to enhance the therapeutic efficacy of quercetin. Nanomaterials. 2021 Oct 9;11(10):2658.

10 Khursheed R, Singh SK, Wadhwa S, Gulati M, Awasthi A. Enhancing the potential preclinical and clinical benefits of quercetin through novel drug delivery systems. Drug discovery today. 2020 Jan 1;25(1):209-22.

11 Azeem M, Hanif M, Mahmood K, Ameer N, Chughtai FR, Abid U. An insight into anticancer, antioxidant, antimicrobial, antidiabetic and anti-inflammatory effects of quercetin: A review. Polymer Bulletin. 2023 Jan;80(1):241-62.

12 Chittasupho C, Manthaisong A, Okonogi S, Tadtong S, Samee W. Effects of quercetin and curcumin combination on antibacterial, antioxidant, in vitro wound healing and migration of human dermal fibroblast cells. International journal of molecular sciences. 2021 Dec 23;23(1):142.

13 Gokhale JP, Mahajan HS, Surana SJ. Quercetin loaded nanoemulsion-based gel for rheumatoid arthritis: In vivo and in vitro studies. Biomedicine & Pharmacotherapy. 2019 Apr 1;112:108622.

14 Yadav BK, Kaur J, Kumar N, Vyas M, Bashary R, Mittal A, Haneef J, Khatik GL. Quercetin as an important nutraceutical and medicinal agent. Plant Archives. 2020;20(2):2537-47.

15 Chittasupho C, Manthaisong A, Okonogi S, Tadtong S, Samee W. Effects of quercetin and curcumin combination on antibacterial, antioxidant, in vitro wound healing and migration of human dermal fibroblast cells. International journal of molecular sciences. 2021 Dec 23;23(1):142.

16 Attar ES, Chaudhari VH, Deokar CG, Dyawanapelly S, Devarajan PV. Nano drug delivery strategies for an Oral bioenhanced quercetin formulation. European Journal of Drug Metabolism and Pharmacokinetics. 2023 Sep;48(5):495-514.

17 Mallya R, Patil K. Recent Developments in Formulation Design of a Multifunctional Phytochemical Quercetin: A Review. Pharmacognosy Reviews. 2021 Jan 1;15(29).

Disclaimers	
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