

Original Research Article

Comparative histological and biochemical evaluation of phenytoin and nifedipine in enhancing wound healing in rat model

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Abstract

Purpose: To investigate the regenerative effects of phenytoin and nifedipine in a full-thickness cutaneous wound model in male rats.

Methods: Rats were randomly and equally divided into 4 groups. Group A was negative control (unwounded skin), group B was positive control (untreated wound), group C received phenytoin (1 % topical application, once daily), and group D received nifedipine (1 % topical application, once daily) for 12 consecutive days. Tissue levels of interleukin-10 (IL-10), tumor necrosis factor- α (TNF- α), vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF) were assessed after 6 and 12 days of treatment. Histopathological examination was performed to evaluate tissue regeneration and wound healing progression.

Results: Phenytoin significantly increased IL-10 and reduced TNF- α compared to control groups ($p < 0.05$). Phenytoin also significantly increased VEGF ($p < 0.05$) and bFGF ($p < 0.05$) compared with the control, reflecting enhanced angiogenic and fibroblast activity. Furthermore, nifedipine significantly increased IL-10 and decreased TNF- α compared to positive control ($p < 0.05$). Phenytoin-treated wounds demonstrated advanced re-epithelialization, organized granulation tissue, and increased extracellular matrix deposition. Treatment with nifedipine showed moderate improvement characterized by reduced inflammation and progressing remodeling.

Conclusions: Phenytoin significantly increased VEGF and bFGF levels compared to control, reflecting enhanced angiogenic and fibroblast activity. These findings support the potential therapeutic role of phenytoin and nifedipine in wound remodelling.

Keywords: Cytokines, Growth factor, Nifedipine, Phenytoin, Wound healing

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INTRODUCTION

The skin represents a primary barrier separating the human body from external environment. It performs vital physiological roles enabling the body to maintain stability and survive despite different environmental conditions that constitute various forms of potential hazards. The skin

protects against harmful stimuli, including mechanical and chemical injuries, microbial invasion, and exposure to various forms of radiation [1]. A cutaneous wound occurs when the integrity of the epidermal layer is damaged. A wound refers to a form of tissue injury that disrupts normal anatomical structure and results in partial or complete loss of function. Tissue regeneration is a crucial step to restore structural

continuity and normal skin function [2]. Wound healing is a complex and well-organized biological process regulated by a network of inflammatory cytokines and growth factors working together to promote tissue repair and regeneration [3]. During the initial inflammatory stage, pro-inflammatory mediators such as tumor necrosis factor- α (TNF- α) play a central role in initiating the immune response.

Anti-inflammatory cytokines, including interleukin-10 (IL-10), are essential for suppressing excessive inflammation and facilitating transition towards tissue recovery [4]. Furthermore, growth factors that promote angiogenesis and cellular proliferation, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), play crucial roles in the formation of new blood vessels, the stimulation of fibroblast activity, and the synthesis of extracellular matrix components [5]. Imbalance or abnormal regulation of these signaling molecules interferes with normal repair, leading to delayed healing or chronic wound formation.

In the past decade, drug repurposing has accelerated in regenerative medicine, driven by the need for cost-effective therapies with established safety data. Phenytoin, a traditional antiepileptic drug, has been extensively reported to enhance wound healing when applied topically [6]. Phenytoin plays a multifaceted role in wound repair, which includes enhanced wound/granulation healing, fibroblast proliferation, collagen deposition, neovascularisation, and antibacterial properties [7].

Nifedipine is a dihydropyridine-class calcium channel blocker commonly prescribed for chronic stable angina and hypertension [8]. Beyond its cardiovascular indications, it has demonstrated potential benefits in skin wound healing. By blocking calcium influx, nifedipine promotes vasodilation and enhanced local blood flow, which improve oxygen and nutrient supply to damaged tissues. Furthermore, it has been shown to modulate vasodilatory action, increase vascular proliferation and reduce collagen in a pig model [9]. However, comparative experimental data evaluating the modulatory effects of phenytoin and nifedipine on inflammatory and angiogenic biomarkers across different stages of wound healing remain limited. Therefore, this study investigated the effects of phenytoin and nifedipine on wound healing and tissue regeneration in a male rat model.

EXPERIMENTAL

Animals

A total of 40 adult male Wistar albino rats (250 – 300 g) were randomly assigned to four groups (10 rats/group), each subdivided into two subgroups ($n = 5$) according to time of assessment (day 6 and day 12). The animals were kept in plastic cages and allowed to acclimatize for 1 week under controlled environmental conditions (25 ± 2 °C, 12-hour light/dark cycle, and ambient humidity). Ethical approval was obtained from the Institutional Animal Ethics Committee (approval no. 4-44), and the study was conducted in compliance with the guidelines for the use and care of laboratory animals [10].

Selection criteria

Inclusion criteria

Animals were included if they were adult male Wistar albino rats with body weight between 250 - 300 g, in good general health with no visible skin lesions or systemic illness and had completed a one-week acclimatization period before experimentation.

Exclusion criteria

Rats were excluded if they had pre-existing skin conditions or systemic disease, signs of poor health, or significant weight loss during acclimatization. Female rats and animals outside the specified weight range were also excluded.

Study design

The animals were randomly divided into four groups ($n = 10$). Each group was further subdivided into two subgroups ($n = 5$) corresponding to the two time points (day 6 and day 12) following treatment initiation. Group A was negative control (unwounded skin), group B was positive control (untreated wound), group C received Phenytoin (1 % topical application, once daily), and group D received nifedipine (1 % topical application, once daily) for 12 consecutive days.

Induction of cutaneous wound

Animals were anesthetized using ketamine (60 mg/kg) and xylazine (5 – 7 mg/kg; intramuscular administration). The dorsal skin was shaved and disinfected with 70 % ethanol. A standardized chemical burn injury was induced by topical application of 99 % trichloroacetic acid (TCA) for 10 min, and the site was thoroughly irrigated with

normal saline. Postoperative analgesia was provided using topical lidocaine spray. The TCA application produced a well-demarcated frosted area measuring approximately 2×2 cm, which subsequently formed a keratinized layer that detached spontaneously, resulting in a wound.

Biochemical assessment

Tissue samples were collected on day 6 and 12 following treatment. Tissue levels of TNF- α , IL-10, VEGF, and bFGF were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Sunlong Biotech Co., China) according to the manufacturer's instructions.

Histopathological assessment

Skin specimens were collected on days 6 and 12, fixed in 10 % neutral buffered formalin, processed routinely, embedded in paraffin, sectioned at 4 – 5 μ m, and stained with hematoxylin and eosin (H & E). Histological evaluation was performed blindly under light microscopy to assess inflammatory cell infiltration, fibrosis, necrosis, vascular changes, fibroblast proliferation, angiogenesis, and re-epithelialization.

Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS, version 25.0; SPSS Inc., Chicago, Illinois, USA). Data were expressed as mean $\pm$ standard deviation (SD) and compared using analysis of variance (ANOVA) followed by the LSD Post hoc test. $P < 0.05$ was considered statistically significant.

RESULTS

Biochemical parameters at Day 6

After 6 days, untreated wounds exhibited a significant increase in TNF- α and VEGF levels compared to normal skin ($p < 0.05$). Phenytoin treatment significantly elevated VEGF levels and showed a non-significant decrease in TNF- α compared to group B ($p > 0.05$). Furthermore, Nifedipine significantly increased TNF- α levels ($p < 0.05$), with no significant increase in VEGF levels compared to group B ($p > 0.05$). Levels of IL-10 and bFGF showed no significant difference among the studied groups ($p > 0.05$; Figure 1).

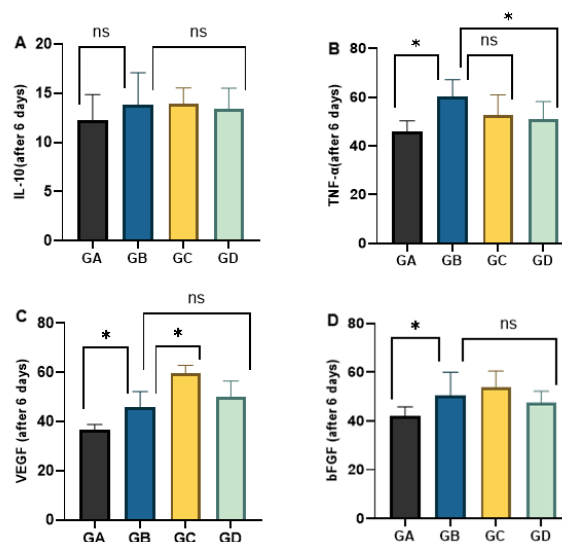


Figure 1: Tissue biochemical parameter levels after 6 days of treatment. (A) IL-10, (B) TNF- α , (C) VEGF, and (D) bFGF. Negative control (GA), Positive control (GB), Phenytoin-treated group (GC), Nifedipine-treated group (GD). * $P < 0.05$

Biochemical parameters at Day 12

After 12 days, untreated wounds demonstrated significantly elevated IL-10, TNF- α , and VEGF ($p < 0.05$), with no significant difference in bFGF levels compared to normal skin ($p > 0.05$). Phenytoin treatment resulted in a significant decrease in TNF- α levels ($p < 0.05$), accompanied by significant increase in IL-10, VEGF, and bFGF compared to group B ($p < 0.05$). Nifedipine treatment also significantly increased IL-10 and reduced TNF- α levels ($p < 0.05$), with no significant difference in VEGF and bFGF levels compared to the untreated group ($p > 0.05$; Figure 2).

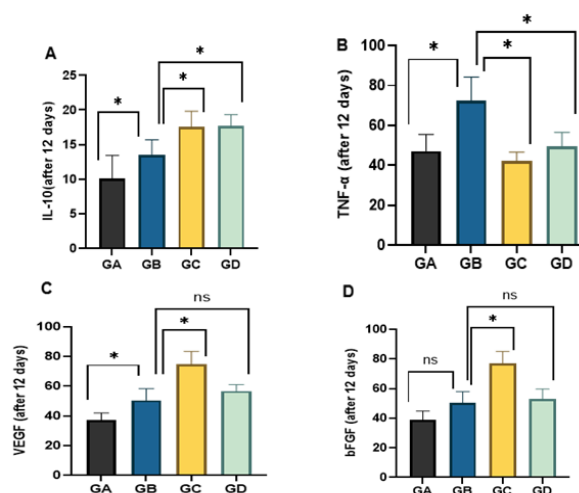


Figure 2: Tissue biochemical parameter levels after 12 days of treatment. (A) IL-10, (B) TNF- α , (C) VEGF, and (D) bFGF. Negative control (GA), Positive control (GB), Phenytoin-treated group (GC), Nifedipine-treated group (GD). * $P < 0.05$

(GB), Phenytoin-treated group (GC), Nifedipine-treated group (GD). * $P < 0.05$

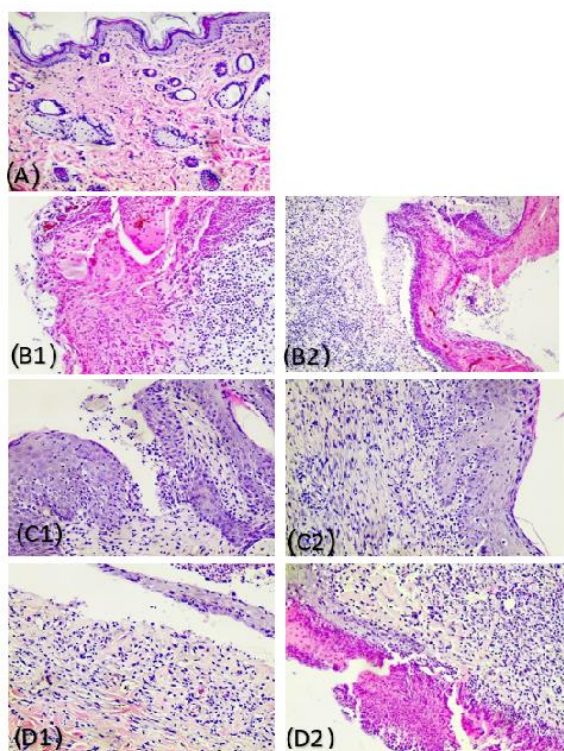


Figure 3: Histological evaluation of wound healing across experimental groups (x100). Group A (negative control), group B (positive control), group C (phenytoin-treated), group D (nifedipine-treated) evaluated at 6 and 12 days after treatment

Histopathological features

Histological sections were evaluated at day 6 and day 12 after treatment across all experimental groups. Group A showed normal skin histology, with an intact epidermis composed of stratified squamous epithelium with multiple layers of keratinocytes, underlain by dermis containing regularly distributed connective tissue and hair follicles. In Group B, after 6 days of treatment (B1), skin tissue showed sloughing of the epidermis and replacement by fibrin clot. The wound bed was dominated by pronounced inflammatory cell infiltration with absent or minimal re-epithelialization. After 12 days (B2), partial epithelial coverage was observed; however, the dermal architecture remained disorganized with persistent inflammatory infiltration. Group C, after 6 days (C1), showed early epithelial migration and granulation tissue formation with fibroblastic activity in the upper dermis, alongside moderate inflammatory cell infiltration. After 12 days (C2), the tissue showed near-complete re-epithelialization, reduced inflammation, and more organized granulation tissue with extracellular matrix deposition consistent with early

remodeling. In Group D, after 6 days (D1), the treatment showed cellular proliferation within the wound bed with moderate inflammatory infiltration. After 12 days (D2), a predominance of mononuclear inflammatory cells with fewer neutrophils was observed, indicative of transition from acute inflammation toward the remodeling phase (Figure 3).

Macroscopic evaluation of wound healing

The macroscopic appearance of cutaneous wounds across experimental groups was evaluated on days 1, 6, and 12 after treatment. On day 1, all wounded groups presented with comparable open TCA-induced chemical-burn wounds of similar appearance and extent. Group B exhibited a persistent open wound with slow contraction over time, with minimal change observed at day 6 and incomplete closure at day 12. Group C demonstrated progressive wound reduction with organized crust formation at day 6 and near-complete closure at day 12. Group D showed limited wound contraction with persistent open wound area at day 6, followed by visible improvement at day 12. However, wound closure remained less advanced compared to group C (Figure 4).

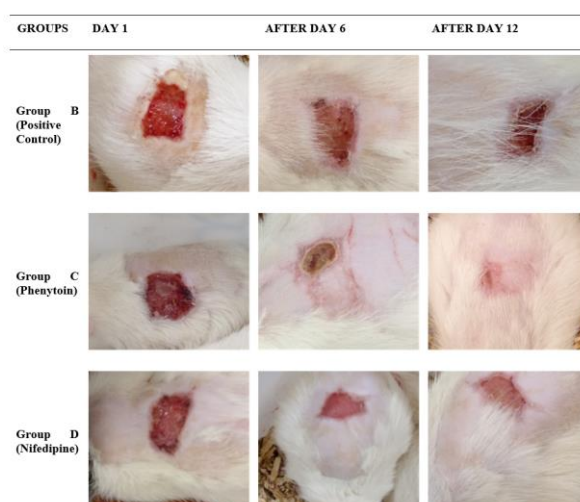


Figure 4: The morphological features of the wound healing progress

DISCUSSION

Wound healing is a highly coordinated process involving four overlapping phases, including hemostasis, inflammation, proliferation and tissue remodeling. Assessment of wound healing may involve different criteria, including epithelialization and reduction in wound size [11]. The balance between pro-inflammatory cytokines and growth factors is critical for successful tissue regeneration [3]. In the present study, the

temporal changes observed in TNF- α , IL-10, VEGF, and bFGF levels reflect the dynamic interaction between inflammation resolution and regenerative signaling. TNF- α is a key mediator of the early inflammatory phase of wound healing, promoting leukocyte recruitment and activation [12]. In this study, untreated wounds showed a significant increase in TNF- α levels at both day 6 and day 12 compared to normal skin, indicating a prolonged inflammatory response. Prolonged inflammatory activity may contribute to delayed wound closure and impaired tissue repair [13]. Furthermore, IL-10 (an anti-inflammatory cytokine) contributes to the regulation of inflammation and tissue repair [14]. At day 6, IL-10 levels showed no significant difference among groups, suggesting that inflammation was still dominant at this early stage. However, by day 12, IL-10 levels were significantly increased in the positive and all treated groups compared with the normal group, with higher mean values observed in the treated groups. This finding may indicate progression toward resolution of inflammation [14]. In the treated groups, the reduction in TNF- α levels was accompanied by an increase in IL-10 levels at day 12. VEGF is a central regulator of angiogenesis and vascular permeability, essential for oxygen and nutrient delivery to healing tissues [15,16]. The significant increase in VEGF levels in untreated wounds at days 6 and 12 was observed in the present study. The enhanced VEGF expression following phenytoin treatment coincided with reduced TNF- α and increased IL-10 levels at day 12, suggesting that angiogenesis was occurring in a more regulated, regenerative environment rather than as a response to chronic inflammation.

Nifedipine showed a higher mean VEGF level than the untreated group, although the difference was not statistically significant, suggesting a possible trend toward increased angiogenic activity [17]. Also, bFGF plays a critical role in fibroblast proliferation, collagen synthesis, and extracellular matrix remodeling [5]. In the early phase, bFGF levels did not differ significantly among groups, indicating limited fibroblast activation during the inflammatory stage. This finding suggests that bFGF expression typically increases during the proliferative phase rather than early inflammation [18]. At day 12, phenytoin treatment resulted in a significant increase in bFGF levels, while nifedipine produced a non-significant increase in bFGF levels. The significant increase in bFGF following phenytoin treatment correlates with its strong VEGF induction and effective suppression of TNF- α , suggesting enhanced granulation tissue formation and matrix deposition. The present

histological findings demonstrated a clear difference in wound healing architecture between untreated and treated groups. The untreated group showed delayed tissue repair characterized by persistent inflammation, fibrin deposition, and poor tissue organization, indicating a prolonged inflammatory phase. This is consistent with the significantly elevated TNF- α levels recorded in Group B at days 6 and 12, suggesting that persistent TNF- α elevation impairs tissue organization and delays wound closure. In contrast, treated groups exhibited enhanced healing with improved granulation tissue formation, fibroblast activity, and progressive re-epithelialization, consistent with previous findings on the role of growth factors in tissue repair [5,18]. At day 12, the significant reduction in inflammatory cells, near-complete re-epithelialization, and extracellular matrix deposition suggested successful transition toward tissue repair and early remodeling. This is consistent with the significant increase in VEGF and bFGF and decrease in TNF- α observed biochemically. These histological observations are consistent with the known effects of phenytoin in stimulating fibroblast activity, promoting angiogenesis, and enhancing extracellular matrix synthesis, thereby accelerating granulation tissue formation and wound contraction [7]. At day 12 following nifedipine treatment, the predominance of mononuclear inflammatory cells with fewer neutrophils indicated the resolution of acute inflammation and progression toward the remodeling phase, consistent with the significant reduction in TNF- α and elevation in IL-10 levels. This may be attributed to the pharmacological action of nifedipine as a calcium channel blocker, which enhances microcirculation, modulates inflammatory signaling, and supports tissue regeneration [17].

CONCLUSION

Phenytoin significantly increased VEGF and bFGF levels compared to control, reflecting enhanced angiogenic and fibroblast activity compared to Nifedipine.

DECLARATIONS

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Use of Artificial intelligence/Large language models

The authors confirm that AI-based tools were used solely for language editing purposes (e.g., grammar, phrasing, and clarity). No AI tools were used to generate content, interpret data, or influence study design, results, or conclusions. All intellectual contributions, study analyses, and interpretations reported in the manuscript are the original work of the authors.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Use of research reporting tools

The study was conducted and reported in accordance with the ARRIVE guidelines for reporting animal research.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Asmaa S. Alrubaei: study conception and design, experimental work, data collection, data analysis, and manuscript preparation. Majid K. Bashara: supervision, study design, and critical revision of the manuscript. Wisam A. Ameen: histopathological evaluation and critical revision of the manuscript. All authors approved the final version of the manuscript

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