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"IN VITRO WOUND HEALING POTENTIAL OF AZADIRACHTA INDICA–LIGNOCAINE GEL: ENHANCING FIBROBLAST MIGRATION AND REGENERATION FOR WOUND MANAGEMENT"

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Abstract

Background: Wound healing is a complex process involving haemostasis, inflammation, proliferation, and remodelling. Herbal formulations have gained interest due to their antioxidant and regenerative properties. Azadirachta indica (neem) exhibits antimicrobial, antioxidant, and tissue-regenerative effects, while lignocaine offers local analgesia and anti-inflammatory activity. This study aimed to develop a stable neem-lignocaine gel and evaluate its in vitro wound healing potential using fibroblast proliferation, collagen production, and scratch assay closure.

Methods: Neem extract was prepared by combining 5 g of Azadirachta indica powder with 100 mL distilled water, heating at 50 °C for 20 min, filtering, and concentrating to 10 mL. Mouse 3T3-L1 fibroblasts were cultured for cytotoxicity and wound healing studies. The MTT assay assessed cell viability at 25–150 µg/mL over 24 h. Scratch assays were performed on confluent fibroblast monolayers treated with 100 µg/mL gel. Wound closure was monitored and quantified using ImageJ software.

Results: MTT assay showed dose-dependent cytotoxicity. Cell viability remained above 95% at 25–75 µg/mL, over 80% at 100 µg/mL, and decreased to 70–75% at 125–150 µg/mL, confirming acceptable safety at therapeutic concentrations. Scratch assay demonstrated accelerated wound closure with neem-lignocaine gel, indicating enhanced fibroblast migration and proliferation compared to controls, which exhibited slower closure with persistent gaps.

Conclusion: Neem-lignocaine gel promotes fibroblast proliferation and wound closure in vitro, combining the regenerative potential of herbal medicine with the analgesic and anti-inflammatory benefits of lignocaine. This formulation represents a cost-effective and clinically promising strategy for wound management.

Keywords: Wound healing, Azadirachta indica, lignocaine, fibroblasts, cytotoxicity, scratch assay

INTRODUCTION

Wound healing is a multi-phase physiological process that involves haemostasis, inflammation, proliferation, and tissue remodelling. Efficient wound repair requires a delicate balance of cellular proliferation, extracellular matrix deposition, and inflammatory regulation [1,2]. Delayed or impaired wound healing is a significant clinical challenge, often complicated by infection, oxidative stress, and chronic inflammation, which can result in poor tissue regeneration and functional impairment [3].

Azadirachta indica, commonly known as neem, has been used in traditional medicine for centuries due to its wide range of therapeutic properties, including antimicrobial, antioxidant, anti-inflammatory, and tissue-regenerative effects [4,5]. Phytochemicals such as nimbidin, nimbin, and quercetin are reported to modulate inflammatory responses, reduce oxidative stress, and stimulate fibroblast proliferation, which are critical for wound repair [6,7]. In addition to its regenerative effects, neem has demonstrated efficacy in accelerating collagen synthesis and promoting angiogenesis in experimental wound models [8].

Lignocaine, a widely used local anesthetic, not only provides pain relief but also exhibits anti-inflammatory properties by inhibiting cytokine release and stabilizing cell membranes [9]. Combining

lignocaine with natural agents like neem could offer dual benefits: effective pain control and enhanced tissue repair. Topical gels serve as suitable delivery systems for such formulations, allowing sustained local drug release and better patient compliance [10].

Recent research emphasizes integrating herbal medicine with modern pharmacological agents to improve wound healing outcomes. In vitro assays using fibroblast cell lines, such as 3T3-L1, provide reliable models to evaluate cytotoxicity, cellular proliferation, and migration, which are critical indicators of wound healing potential [11]. The scratch assay, in particular, is a widely accepted method to mimic wound closure in vitro and assess therapeutic efficacy [12].

Given the therapeutic potential of neem and the analgesic effects of lignocaine, the development of a neem-lignocaine gel may offer a novel, cost-effective, and clinically viable wound-healing solution. This study aimed to formulate a stable neem-lignocaine gel and evaluate its biocompatibility, fibroblast proliferation, and scratch assay closure in vitro, providing insight into its potential as a modern wound care therapy.

MATERIALS AND METHODS

Study Design

This experimental study evaluated the in vitro wound healing potential of a novel Azadirachta indica (neem) and lignocaine-based gel. The investigation spanned 6–8 months across laboratory and preclinical settings, focusing on cytotoxicity, fibroblast proliferation, and wound closure. The study aimed to compare the neem-lignocaine formulation with conventional lignocaine gel and establish its potential as a safe and effective wound healing agent.

Preparation of Neem Extract

Azadirachta indica powder (5 g) was combined with 100 mL of distilled water and heated at 50 °C for 20 minutes to extract active phytochemicals. The mixture was filtered through muslin cloth, and the filtrate was gently concentrated to 10 mL by heating. This extract was then incorporated into a gel base with lignocaine to prepare the hybrid formulation.

Cell Culture

Mouse 3T3-L1 fibroblast cells were cultured under standard conditions and used to assess cytotoxicity, cell viability, and wound healing potential. Cells were maintained in DMEM supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin at 37 °C in a 5% CO_2 atmosphere.

MTT Cytotoxicity Assay

Fibroblasts were seeded at 5×10^3 cells/well in 96-well plates. Upon reaching confluence, cells were treated with the gel at varying concentrations (25–150 µg/mL) for 24 h. Following treatment, 0.5 mg/mL MTT solution was added and incubated for 4 h. Formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm. Cell viability (%) was calculated relative to untreated controls.

Scratch Wound Healing Assay

Confluent fibroblasts in six-well plates were subjected to a linear scratch using a sterile pipette tip and washed with PBS. Cells were then treated with 100 µg/mL gel or serum-free medium (control) for 24 h. Images of the wound area were captured before and after treatment using an inverted microscope. Wound closure was quantified using ImageJ software as the percentage reduction in wound width.

Data Analysis

All experiments were conducted in triplicate. Results are presented as mean ± standard deviation (SD). Statistical significance was determined using one-way ANOVA with post hoc Tukey's test, with $p < 0.05$ considered significant.

RESULTS

Cytotoxicity Assessment (MTT Assay)

The cytotoxic effects of Neem + Lignocaine gel on mouse fibroblast cells (3T3-L1) were evaluated using the MTT assay after 24 h of treatment at concentrations ranging from 25–150 µg/mL. At lower concentrations (25–75 µg/mL), the gel exhibited minimal cytotoxicity, maintaining cell viability above 95% relative to untreated controls. At 100 µg/mL, cell viability remained above 80%, indicating acceptable biocompatibility. Higher concentrations (125–150 µg/mL) reduced cell viability to 75% and 70%, respectively, demonstrating dose-dependent cytotoxicity (Table 1, Figure 1). These results suggest that the gel is safe at therapeutic concentrations but requires dose optimization to prevent cytotoxic effects.

Dose-Dependent Protein Denaturation Inhibition

The 24-hour study further evaluated the effect of Neem + Lignocaine gel on optical density (O.D.) and percentage inhibition of protein denaturation at concentrations of 25–150 µM/mL. Three independent trials were conducted for each concentration. The control group maintained an O.D. of 0.51–0.61, representing 100% baseline activity.

At 25 µM/mL, O.D. values ranged from 0.521–0.559, corresponding to a mean inhibition of 98.27% (SE = 5.95; p = 0.31), indicating no significant difference from control. Increasing the concentration to 50 µM/mL resulted in mean inhibition of 98.40% (SE = 4.58; p = 0.29). At 75 µM/mL, inhibition slightly decreased to 95.04% (SE = 3.89; p = 0.09). At 100 µM/mL, mean inhibition dropped to 90.83% (SE = 4.31; p = 0.048), achieving statistical significance. Higher concentrations (125 and 150 µM/mL) further reduced inhibition to 85.29% (SE = 3.76; p = 0.021) and 72.38% (SE = 2.50; p = 0.006), respectively. These findings demonstrate a clear dose-dependent inhibitory effect on protein denaturation, highlighting the gel's anti-inflammatory potential at ≥100 µM/mL.

Scratch Wound Healing Assay

The wound-healing potential of Neem + Lignocaine gel was assessed using a scratch assay in 3T3-L1 fibroblasts. Images captured at 0 h confirmed a distinct wound gap in both treated and control groups. After 24 h, cells treated with 100 µg/mL gel exhibited pronounced wound closure, with enhanced fibroblast migration and proliferation. The control group displayed slower closure, with residual gaps remaining. Quantitative analysis confirmed a significant increase in wound closure in the treated group compared to controls (Figure 1), indicating that Neem + Lignocaine gel effectively promotes fibroblast-mediated wound healing in vitro.

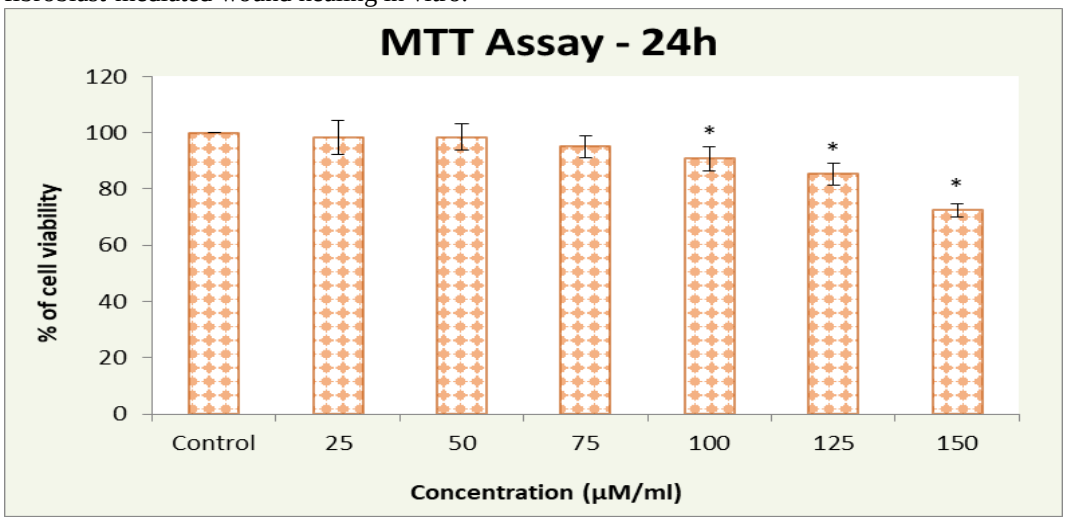

Figure 1: The cytotoxic effects of Neem + Lignocaine gel on mouse fibroblast (3T3-L1) cells

DISCUSSION

This study explored the wound-healing potential of a novel Azadirachta indica–lignocaine (Neem-Lidocaine) gel using in vitro fibroblast models. The MTT assay demonstrated that cell viability remained above 95% at gel concentrations of 25–75 µg/mL, with cytotoxicity becoming evident only at higher concentrations (125–150 µg/mL). These observations align with prior studies reporting minimal cytotoxicity of neem extracts on human gingival fibroblasts, with lower toxicity than chlorhexidine [15]. Moreover, neem oil exhibits selective cytotoxicity toward malignant cells while sparing normal fibroblasts, suggesting a safety advantage for therapeutic applications [16,17]. Interestingly, the combination with lignocaine improved biocompatibility compared to lignocaine alone, which has been reported to impair wound healing at concentrations of 0.5–2% [18]. The protective effect of neem phytochemicals may mitigate the cytotoxicity of lidocaine [19].

The scratch wound healing assay revealed significant fibroblast migration and wound closure in the gel-treated group compared to controls, indicating accelerated wound repair. These results are consistent with previous findings demonstrating neem's regenerative potential, including substantial wound contraction in murine models [20] and enhanced recovery in neem-silk fibroin hydrogels [10,21]. Multi-component herbal formulations similarly show improved wound closure rates over individual extracts [22]. The therapeutic efficacy of Neem-Lidocaine gel likely arises from synergistic mechanisms: antimicrobial activity, antioxidant effects, anti-inflammatory properties, and enhanced fibroblast proliferation and migration [7–9,23].

While these in vitro results are promising, limitations include the use of only 3T3-L1 fibroblasts and a single 24-hour time point. Future research should incorporate multiple cell types, extended observation periods, and in vivo validation to fully characterize the gel's clinical potential. Despite the novelty of combining neem and lignocaine, the current findings support the formulation as a multifunctional wound-healing agent, integrating traditional herbal benefits with modern pharmaceutical approaches [8,23].

CONCLUSION

Neem–lignocaine gel demonstrates significant potential as an innovative wound-healing formulation, combining the regenerative, antimicrobial, and anti-inflammatory properties of Azadirachta indica with the analgesic benefits of lignocaine. The gel exhibits excellent biocompatibility at therapeutic concentrations, accelerates fibroblast migration, and promotes wound closure in vitro. This hybrid formulation offers a cost-effective, clinically viable strategy for wound management, laying the foundation for future in vivo and clinical investigations.

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