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Galaxy Publication

Therapeutic Potential of Himatanthus drasticus Latex Proteins in Wound Healing: Modulation of Inflammatory and Proliferative Phases

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ABSTRACT

This study explored the wound-repair effects of proteins isolated from the latex of *Himatanthus drasticus* (HdLP) using a murine excisional wound model. Toxicity tests revealed that HdLP, at concentrations between 12.5 and 100 μg/ml, was safe for fibroblast cells, and dermal irritation assays confirmed it did not provoke adverse skin reactions. Wounds were treated with HdLP at 0.5%, 1.0%, and 2.0%, or with a vehicle control, and healing was assessed through gross observation, histology, and analysis of inflammatory markers. Treatment with HdLP triggered an early increase in IL-1β during the inflammatory phase, which appeared to support a timely rise in IL-10 during the proliferative phase. Enhanced fibroblast proliferation, deposition of new collagen fibers, and orderly re-epithelialization contributed to faster and more complete wound closure. These findings provide the first evidence that latex-derived proteins from *Himatanthus drasticus* actively participate in tissue remodeling and wound healing.

Keywords: Wound repair, Tissue regeneration, *Himatanthus drasticus*, Latex proteins

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Introduction

Plant latex is a complex biological fluid containing soluble molecules, ions, proteins, subcellular structures, and other cellular components, which are synthesized or stored by specialized cells known as laticifers [1]. Unlike typical plant tissues, laticifers do not belong to specific organs; they develop through elongation of cells and form extensive tubular networks throughout the plant body, spanning multiple tissues and organs [2]. Latex accumulates in these structures and is released when the plant is physically damaged. Accumulating evidence highlights the diverse defensive roles of latex in plants [3]. Many latex-producing species are utilized in traditional medicine and are described in classical pharmacopoeias such as Ayurveda [4, 5]. Extracted latex from these plants exhibits a variety of pharmacological, toxicological, and biological activities [6–8], with certain compounds being investigated for anticancer or antimetabolic properties [9–11]. Recent studies have emphasized the potential of latex and its constituents to prevent or treat various pathological conditions in humans [12, 13].

Himatanthus drasticus (Mart.) Plumel, belonging to the Apocynaceae family, is a tree whose latex is primarily obtained from the bark. In northeastern Brazil, this latex—locally known as "Janaguba"—is commonly sold in street markets and widely used in folk medicine to manage inflammatory disorders and conditions such as cancer, diabetes, gastritis, ulcers, hepatitis, and parasitic infections [14, 15]. Experimental studies have also supported its antidiabetic and gastroprotective effects [16–18], and ethnopharmacological surveys indicate its traditional use in treating gastric ulcers [19].

Despite these observations, the potential of *H. drasticus* latex to promote wound repair remains largely unexplored. Skin wounds provide a convenient and controlled model to monitor the healing process over time, making them suitable for evaluating the biological activity of latex. The specific latex components responsible for any observed wound-healing effects are still unknown, prompting further investigation.

Wound healing is a coordinated physiological process aimed at restoring tissue integrity, typically progressing through three overlapping stages: inflammation, proliferation, and remodeling. The initial inflammatory phase, occurring within the first 48 hours, is characterized by infiltration of immune cells and the release of cytokines such as IL-1β, TNF-α, and IFN-γ, resulting in edema and hyperemia observable by day two. The proliferative phase begins around day two and continues until day ten, during which fibroblast proliferation, angiogenesis, collagen deposition, and re-epithelialization occur, leading to contraction of the wound and partial restoration of tissue structure. The final remodeling phase starts approximately two weeks after injury and can last for months to years, during which the extracellular matrix matures and scar tensile strength increases, ultimately restoring normal tissue architecture [20].

In this study, latex was collected from taxonomically verified *H. drasticus* plants and processed to isolate its soluble protein fraction, termed HdLP. Topical application of HdLP on surgically induced excisional wounds in mice demonstrated enhanced tissue organization and accelerated healing, as confirmed by histological analyses.

Materials and Methods

Ketamine hydrochloride and xylazine hydrochloride were obtained from Vetbrands (São Paulo, Brazil), iodopovidone from ADV (São Paulo, Brazil), and hematoxylin–eosin from Merck Millipore (São Paulo, Brazil). All other reagents were of analytical grade unless stated otherwise.

Specimens of *Himatanthus drasticus* (Mart.) Plumel (Apocynaceae) used for latex extraction were collected at coordinates 3°46′58.81″S/38°27′59.42″W, forming a unique local population. The botanical identity of the species was verified in April 2020 using The Plant List (www.theplantlist.org). Locally, the plant is known as "Janaguba," a name consistent with the literature in English. Voucher specimens were deposited and examined at the Institutional Herbarium of the Federal University of Ceará under the reference code 40408. Legal authorization to access and study the species was obtained from SisGen (Genetic Heritage and Associated Traditional Knowledge System, Brazil) in compliance with national regulations (access code A689147). The latex samples used were the same as those described in Morais *et al*. [16], with additional phytochemical details available therein.

Extraction of Himatanthus drasticus latex proteins (HdLP)

Latex proteins were isolated following previously described protocols [9, 16]. Approximately 500 ml of latex was collected from mechanically injured tree trunks. The latex was immediately mixed with an equal volume of distilled water (1:1 v/v) to ensure homogeneity and stored at 8°C until processing. The mixture was centrifuged at 10,000×g for 10 minutes at 4°C to remove insoluble material. The supernatant was dialyzed against distilled water for 72 hours at 8°C using membranes with an 8,000 Da cutoff, with water replaced three times daily to eliminate low-molecular-weight substances. After dialysis, the solution was centrifuged again to remove precipitates. This procedure removed insoluble material, rubber particles, polyisoprenes, starch, and small soluble compounds, while retaining water-soluble proteins. The resulting protein fraction, HdLP, was freeze-dried and stored for subsequent use. HdLP accounted for approximately 3.1% of the dry matter from latex fractionation and was used to prepare topical ointments for wound treatment. Preliminary biochemical and pharmacological characterization of HdLP has been previously reported [15].

Cell culture

Murine fibroblast L929 cells (NCTC clone L929; ATCC) were obtained from the Rio de Janeiro Cell Bank (Brazil). Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM, Gibco®) supplemented with 10% fetal bovine serum (FBS, Gibco®) and antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin, Gibco®) at 37°C in a humidified incubator with 5% CO_2.

Assessment of HdLP cytotoxicity

Cell viability was evaluated using the MTT assay [21]. L929 cells (4×10^3 cells/200 µl/well) were seeded in 96-well plates in DMEM containing 2.5% FBS and incubated for 24 hours (37°C, 5% CO_2). HdLP stock solution (1 mg/ml in sterile water) was filtered through a 0.22 µm membrane and applied at concentrations ranging from 12.5 to 100 µg/ml. Sterile water was used as a control, and doxorubicin (5 µM) served as a positive control. After 72 hours, 20 µl of MTT solution (5 mg/ml) was added and incubated for 3 hours. Formazan crystals were solubilized

in 150 µl dimethyl sulfoxide (DMSO), and absorbance was measured at 540 nm using a microplate reader (Biochrom Elisa Asys Expert Plus). Cell viability was expressed as a percentage relative to the control.

Preparation of HdLP ointment

A water-in-oil (W/O) ointment was formulated to accommodate the hydrophilic protein fraction for topical wound application. HdLP (1 g) was first dissolved in 3 ml distilled water (aqueous phase). Anhydrous lanolin (up to 10% w/v) was gradually added under continuous mixing to emulsify the aqueous solution. The final semi-solid formulation was completed by incorporating polyethylene petrolatum to achieve uniform consistency. Ointments containing 0.5%, 1.0%, and 2.0% HdLP (w/w) were prepared and stored at 8°C until use in excisional wound experiments.

- *Animals*

Adult male Swiss mice (12 weeks old, 25 ± 3 g) were obtained from the Central Animal House of the Federal University of Ceará, Brazil. Animals were maintained under controlled conditions: temperature 25 ± 3°C, humidity 55 ± 10%, and a 12 h light/dark cycle, with ad libitum access to standard chow and water. A total of 168 mice were used. All experimental procedures adhered to the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee (approval number 39/2014).

Animals were randomly allocated into four groups: sham (surgery only, no topical treatment), regederm® (commercial latex protein ointment from *Hevea brasiliensis*), vehicle (ointment without HdLP), and HdLP (ointment containing *Himatanthus drasticus* latex proteins at different concentrations). The vehicle formulation contained all ointment components except the active protein fraction.

- *Skin irritation assay*

Skin irritation was evaluated following previously described methods [22]. Mice (19 ± 1 g) were shaved on a 4 cm² dorsal area and observed for 24 hours. A 1 cm² region was superficially scarified with a scalpel until tissue exudate appeared, avoiding bleeding. Animals were randomly assigned to three groups (n = 6/group): sham, vehicle, or 2.0% HdLP ointment. Approximately 50 mg of ointment was applied daily to the scarified area for six consecutive days. Non-scarified regions of the same size were also treated to assess irritation. Skin thickness was measured with a digital caliper at 1, 2, 4, and 6 hours after application and daily for six days. Dermal reactions, including erythema and edema, were recorded during the first two days. Data were analyzed using ANOVA followed by Tukey's post hoc test.

- *Wound healing assays*

A full-thickness excisional wound model was used to assess wound repair, allowing evaluation of macroscopic changes, inflammatory responses, re-epithelialization, and molecular markers [23]. Mice (n = 10 per group) were anesthetized with intramuscular ketamine (100 mg/kg, 10%) and xylazine (10 mg/kg, 2%). After shaving and antiseptic preparation with 1% iodopovidone and 70% ethanol, a single 1 cm² circular wound was created on the dorsal skin using a punch, reaching the dermo-epidermal layer. Subcutaneous saline (1 ml, 0.9%) was administered post-surgery for fluid replacement, and mice were maintained in individual, ventilated cages for 14 days. Cages were cleaned daily to maintain hygiene. Postoperative care followed protocols described in prior studies [22, 24, 25].

- *Macroscopic analysis*

Wounds were monitored for edema, hyperemia, and contraction on days 2, 9, and 14 (n = 10 per group) [26]. Edema and hyperemia were scored as 0 (absent), 1 (mild), 2 (moderate), or 3 (severe) on day 2. Wound areas were measured using a caliper and calculated using the formula: $A = \pi \times R \times r$, where R and r represent the major and minor radii [25]. At the study endpoint, animals were euthanized under anesthesia, and wound tissue with adjacent normal skin was collected for histological analysis and inflammatory mediator evaluation.

Histological analysis

Histology focused on proliferative phase parameters (n = 5 per group). Wounds treated with 2.0% HdLP were analyzed on days 9 and 14. Tissue samples were fixed in 10% formaldehyde (PBS 0.01 M, pH 7.4) for 24 h, processed, and sectioned at 5 µm. Hematoxylin-eosin staining assessed fibroblast density and re-epithelialization,

while Masson's trichrome staining quantified collagen deposition. Images were captured using a Leica DM microscope with Leica DFC 280 camera. Re-epithelialization was calculated as the percentage of newly formed epithelium relative to total wound area (100× magnification). Collagen deposition was quantified in 15 randomly selected fields (200×, 372 × 272 pixels) using ImageJ®1.46 software, applying "cell counter" and "threshold color" plug-ins [27].

Inflammatory markers and mediators

Inflammatory parameters were assessed in a separate experiment (n = 6 per group, without regederm®). Neutrophil infiltration was estimated indirectly via myeloperoxidase (MPO) activity in wound tissue on days 2 and 3 post-surgery [28]. MPO activity was determined by monitoring O-dianisidine oxidation in the presence of H_2O_2 (460 nm). Nitrite levels in tissue lysates were measured as an indicator of total NO content using the Griess reaction on day 2 [29]. Cytokine levels (TNF-α, IL-1β, IL-6, IL-10) were quantified in tissue homogenates by sandwich ELISA and expressed as pg/ml.

Statistical analysis

Results are presented as mean ± SEM or median values, depending on the data type. Comparisons between multiple groups were performed using ANOVA followed by Tukey's post hoc test. Median comparisons were analyzed using Kruskal-Wallis and Dunn's tests. Statistical significance was set at $P < 0.05$. Analyses were conducted using GraphPad Prism version 5.0 (USA).

Results and Discussion

HdLP did not exhibit cytotoxic effects on L929 murine fibroblasts after 72 hours of incubation, even at the highest concentration tested (100 µg/ml), when compared to untreated controls **(Figure 1)**. In the dermal irritation assay, no visible reactions such as edema or erythema were observed, and skin thickness in both scarified and non-scarified areas remained similar to the sham group, indicating that HdLP (2.0%) and the vehicle were well tolerated.

Representative macroscopic images of wound healing across all treatment groups and controls are shown in **Figure 2a**. Day 2 images reflect the early inflammatory phase, with edema and hyperemia evident in all groups. Notably, wounds treated with 2.0% HdLP exhibited reduced edema (ranging from absent to mild) compared to the sham group, which showed mild to moderate edema ($P < 0.05$). Hyperemia did not differ significantly among groups **(Table 1).**

By days 9 and 14, corresponding to the proliferative and remodeling phases, wounds treated with 2.0% HdLP demonstrated accelerated healing and more uniform scar formation by day 14. HdLP (2.0%) significantly enhanced wound contraction compared to controls after 9 days ($P < 0.05$), **(Figure 2b),** which likely contributed to the complete re-epithelialization and scar maturation observed at the final time point. Lower concentrations of HdLP (0.5% and 1.0%) did not produce significant differences in edema, hyperemia, or wound area reduction compared with sham, vehicle, or regederm® groups. Therefore, only the 2.0% HdLP formulation was selected for subsequent detailed analyses.

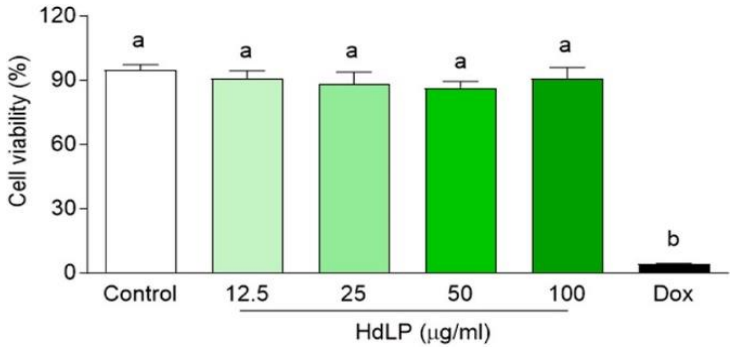


Figure 1. Assessment of L929 fibroblast viability after 72 hours of incubation with HdLP at concentrations ranging from 12.5 to 100 µg/ml, using the MTT assay. The control group (vehicle) received sterile water, the same used to dissolve HdLP. Doxorubicin (5 µM) served as a positive control. Three independent experiments were conducted (n = 4 per group per experiment). Data are presented as mean ± standard error of

the mean (SEM). Different letters denote statistically significant differences between groups ($P < 0.001$, Tukey's test).

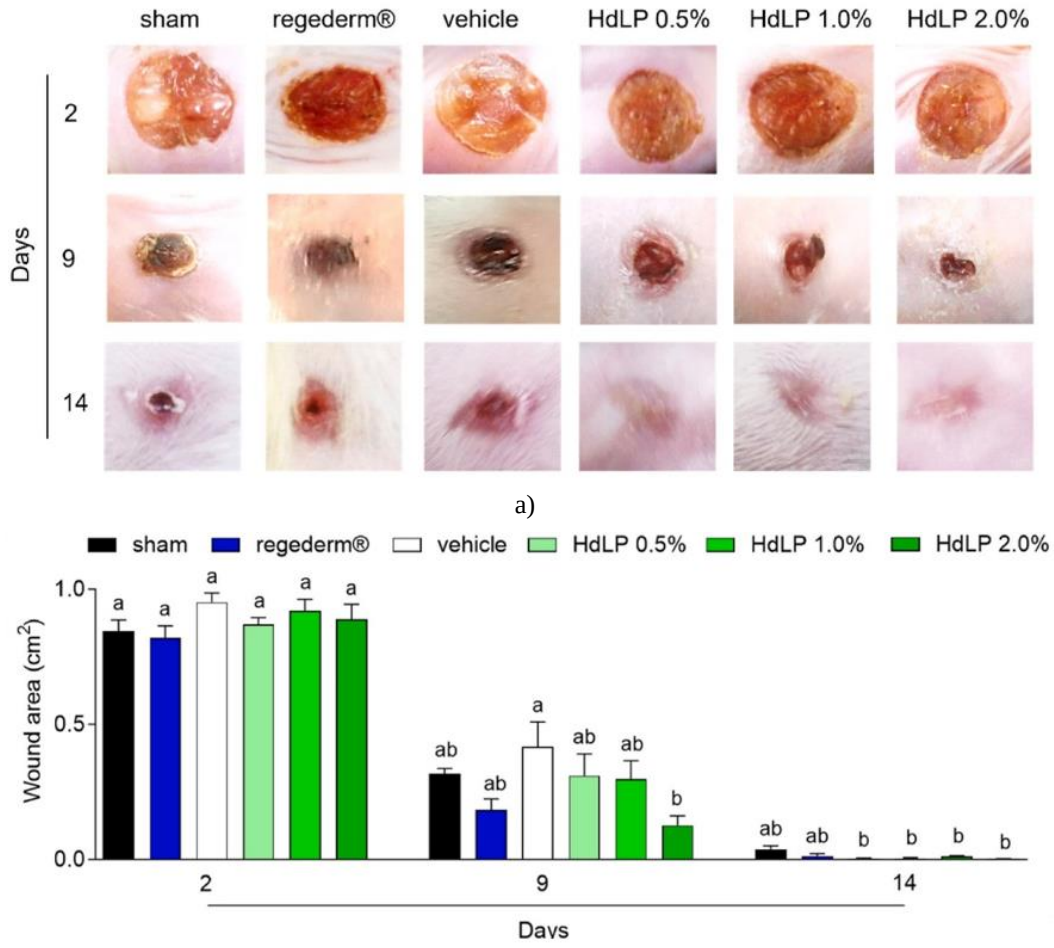


Figure 2. Evaluation of excisional wound healing: (a) macroscopic appearance and (b) wound area (cm²). (a) Representative images from each experimental group: sham (surgery only, no topical treatment), regederm® (positive control), vehicle (ointment without HdLP), and HdLP (ointment containing 0.5%, 1.0%, or 2.0% HdLP). (b) Wound areas were measured using a caliper on days 2, 9, and 14 post-surgery. Data represent the mean ± SEM from three independent experiments. Identical letters indicate no significant difference ($P > 0.05$), while different letters denote statistically significant differences ($P < 0.05$, Tukey's test; $n = 10$ animals per group per time point).

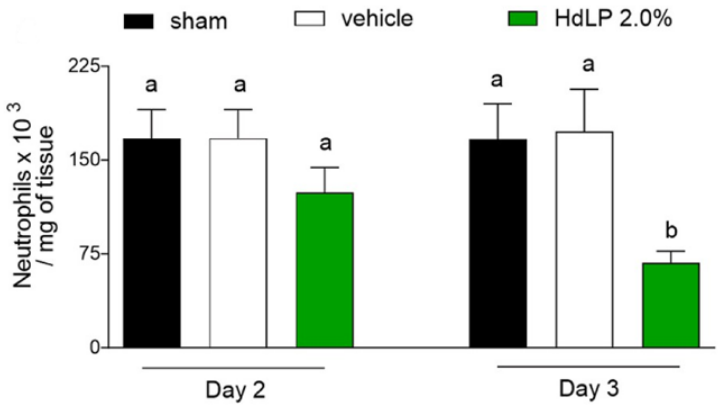
Table 1. Semi-quantitative assessment of edema and hyperemia in excisional wounds treated with HdLP at different concentrations

Parameter	Day	Sham	Regederm®	Vehicle	HdLP 0.5%	HdLP 1.0%	HdLP 2.0%
Edema	2	1 (1–2)^{a}	1 (0–2)^{ab}	1 (0–2)^{ab}	1 (0–2)^{ab}	1 (0–2)^{ab}	1 (0–1)^{b}
Hyperemia	2	1 (1–2)^{a}	1.5 (0–2)^{a}	1 (0–2)^{a}	1 (0–2)^{a}	1 (0–2)^{a}	1 (0–2)^{a}

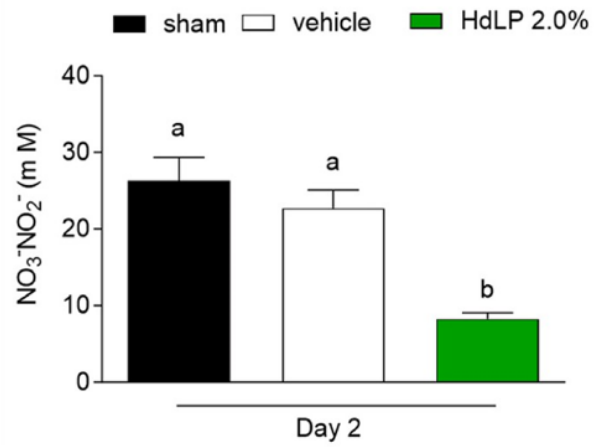
Application of 2.0% HdLP led to a significant decrease in neutrophil MPO activity on day 3 when compared to both the vehicle and sham groups ($P < 0.05$), **(Figure 3a)**. On day 2, nitrite levels, reflecting nitric oxide production, were notably lower in HdLP-treated wounds than in the vehicle ($P < 0.01$) and sham groups ($P < 0.001$), **(Figure 3b).** TNF-α concentrations were not significantly affected by HdLP treatment **(Figure 4a)**. Interestingly, HdLP (2.0%) triggered a marked elevation in IL-1β and IL-10 levels on day 2 relative to sham and vehicle controls ($P < 0.001$), **(Figures 4b and 4c).**

Histological assessment showed that wounds receiving HdLP (2.0%) had a longer re-epithelialized layer on day 9 than those in the sham, vehicle, and regederm® groups ($P < 0.05$), **(Figure 5).** Moreover, fibroblast proliferation

was significantly higher in HdLP-treated wounds on days 9 and 14 compared with sham ($P < 0.05$) and vehicle groups ($P < 0.001$), **(Figure 6a).** Collagen fiber accumulation during tissue remodeling was enhanced by HdLP (2.0%) on day 14, with significantly more deposition than in sham ($P < 0.01$) and vehicle-treated wounds ($P < 0.001$), **(Figure 6b).**

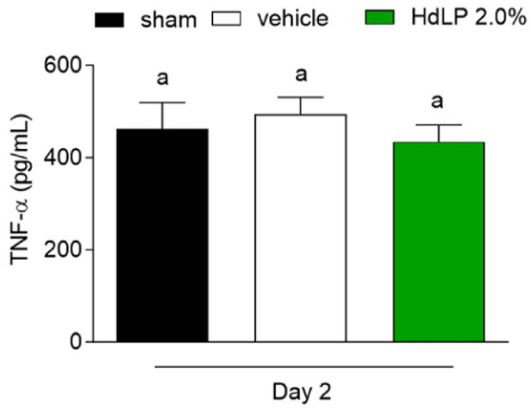


a)

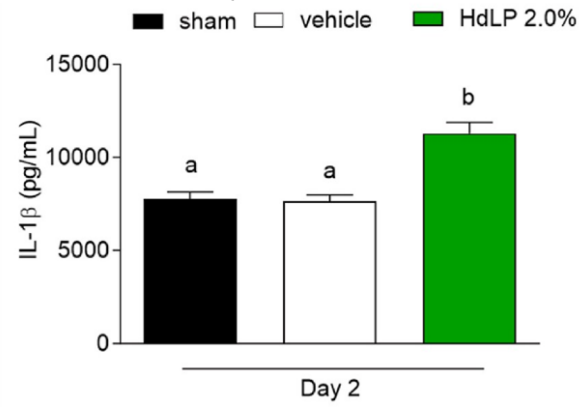


b)

Figure 3. Assessment of neutrophil infiltration (a) and nitrite concentrations (b) in wound tissues on days 2 and 3 post-surgery. Wounds were harvested after euthanasia to measure neutrophil infiltration and nitrite levels using the Griess reaction. Experimental groups included: sham (surgery only, no topical treatment), vehicle (ointment without HdLP), and HdLP (ointment containing 2.0% HdLP). Data are presented as mean ± SEM for neutrophil counts (×10³/mg tissue, a) and nitrite levels (μM, b). Identical letters indicate no significant differences ($P > 0.05$), while different letters denote significant differences ($P < 0.05$, Tukey's test; n = 6 animals per group per time point).



a)



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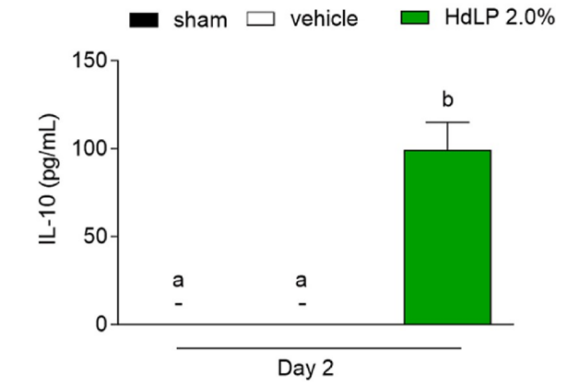


c)

Figure 4. Effects of 2.0% HdLP on cytokine levels: TNF-α (a), IL-1β (b), and IL-10 (c). On day 2 post-surgery, animals were euthanized and wound tissues were collected to quantify cytokine concentrations using ELISA. Experimental groups included sham (surgery only, no treatment), vehicle (ointment without HdLP), and HdLP (ointment containing 2.0% HdLP). Data are presented as mean ± SEM of cytokine levels (pg/ml) per mg of tissue. Matching letters indicate no significant difference ($P > 0.05$), while differing letters denote statistically significant differences ($P < 0.05$, Tukey's test; $n = 6$ animals per group).

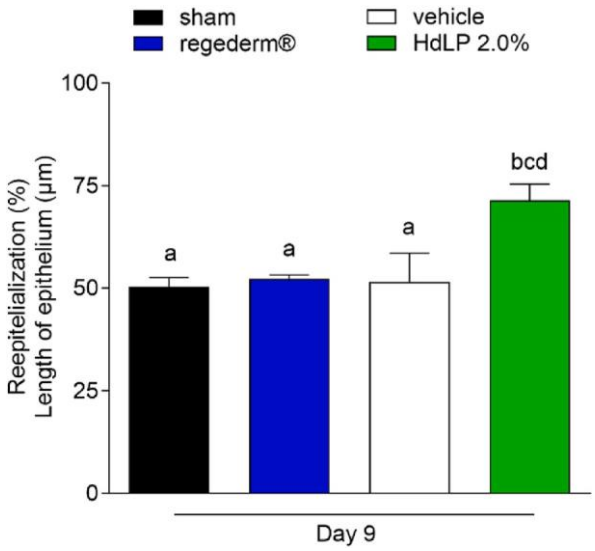
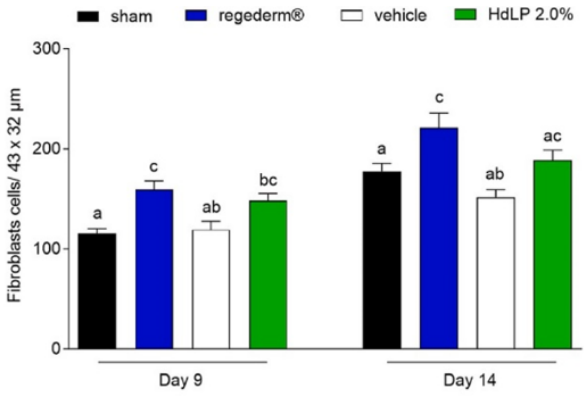
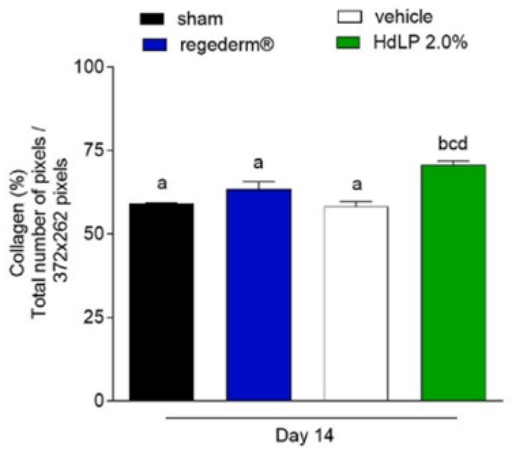


Figure 5. HdLP-induced re-epithelialization. On day 9 post-surgery, animals were euthanized and wound tissue samples were collected for histological evaluation. Experimental groups included sham (surgery only, no treatment), regederm® (positive control), vehicle (ointment without HdLP), and HdLP (ointment containing 2.0% HdLP). The extent of newly formed epithelium was measured using hematoxylin–eosin

staining. Data are presented as mean ± SEM of the percentage of epithelial length. Identical letters indicate no significant differences (P > 0.05), whereas differing letters denote significant differences (P < 0.05, Tukey's test; n = 5 animals per group).



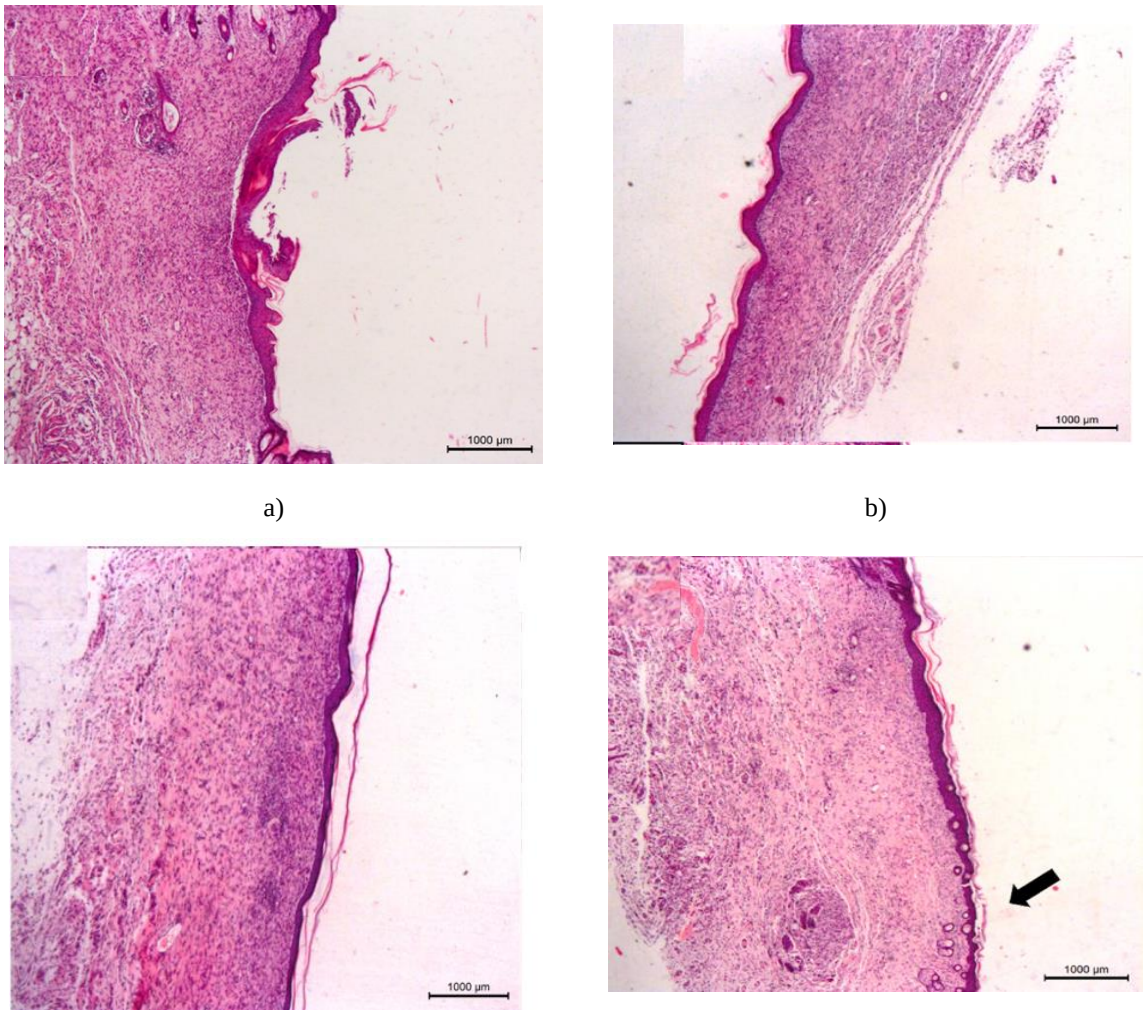
a)



b)

Figure 6. Effects of HdLP on fibroblast proliferation and collagen deposition during wound healing. Tissue samples were collected on days 9 and 14 post-surgery for histological evaluation. Groups included sham (surgery only, no treatment), regederm® (positive control), vehicle (ointment without HdLP), and HdLP (ointment containing 2.0% HdLP). Fibroblast proliferation was assessed using hematoxylin–eosin staining (a), and collagen deposition was evaluated in Masson's trichrome-stained sections (b). Data are expressed as mean ± SEM for fibroblast counts and collagen content (percentage of pixels in a fixed area of 372 × 262 pixels). Matching letters indicate no significant differences (P > 0.05), while different letters indicate significant differences (P < 0.05, Tukey's test; n = 5 animals per group per time point).

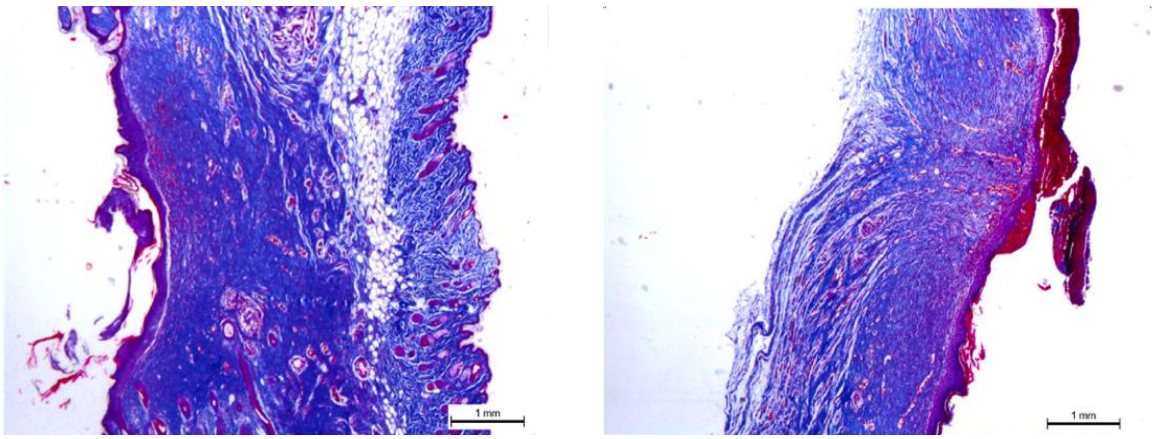
At day 14, sham and vehicle groups showed small ulcerated areas and residual inflammatory cells, whereas HdLP (2.0 %) treatment resulted in enhanced collagen deposition, better tissue architecture, reduced cellularity, and increased epithelial keratinization, comparable to the regederm® group.



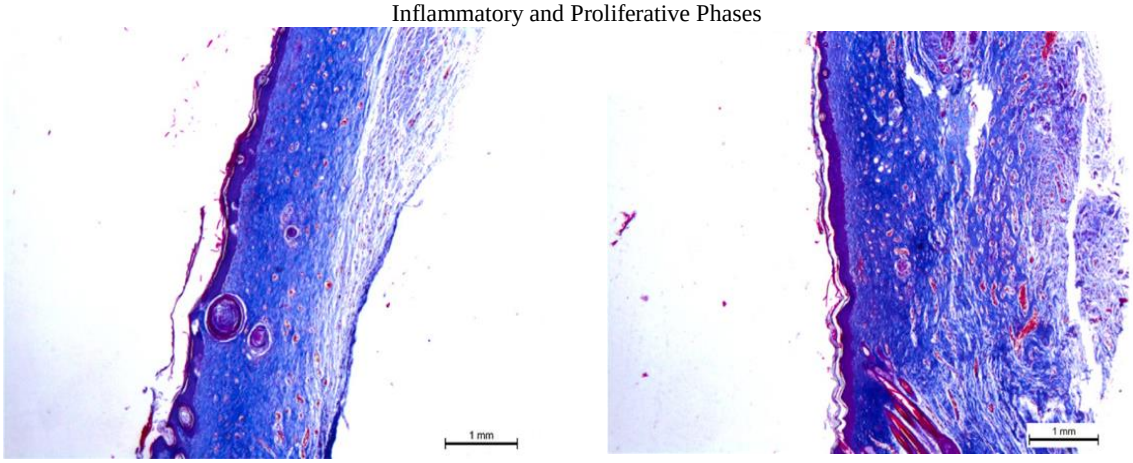
a) b)

c) d)

Figure 7. HdLP promotes complete tissue re-epithelialization 14 days post-surgery. Representative H&E-stained histological sections (40× magnification) showing newly formed epithelium from each experimental group. A black arrow highlights hair follicles observed in the HdLP (2.0 %) treated group. Groups: sham (surgery only, no treatment – a); regederm® (positive control – b); vehicle (ointment without HdLP – c); and HdLP (ointment with 2.0 % HdLP – d).



a) b)



c) d)

Figure 8. Representative histological images of tissue sections stained with Masson's trichrome to assess collagen deposition 14 days after surgery (40× magnification). The group treated with 2.0 % HdLP showed denser collagen fibers, more keratin filaments, and a thicker epidermis compared to other groups (collagen appears blue; keratin appears red). Groups: sham (no topical treatment – a); regederm® (positive control – b); vehicle (ointment without HdLP – c); and HdLP (ointment with 2.0 % HdLP – d). (For interpretation of colors, refer to the Web version of this article.)

There are no reports of toxicity linked to oral consumption of H. drasticus latex. Locally, people often consume the latex diluted 1:1 in water for its presumed preventive or therapeutic properties. This common practice suggests that diluted latex does not cause acute toxicity. However, recent studies have shown that certain latex secondary metabolites may be toxic at very high doses (2000 mg/kg) in mice [7]. Previous work by our group has characterized the chemical composition of H. drasticus latex and identified plumieride, which inhibits α-amylase and α-glucosidase [16]. In this study, all non-protein compounds were removed before testing.

Prior to in vivo experiments, HdLP was evaluated for cytotoxicity in vitro using the MTT assay. HdLP showed no cytotoxic effects even at the highest tested concentration (100 μg/ml), corroborating previous findings [9]. This step ensured safe dosage selection for both in vitro and in vivo studies. Nonetheless, single-exposure in vitro assays provide only limited safety insights, and caution is needed when extrapolating these results.

In the in vivo wound model, HdLP was applied topically daily at a maximum concentration of 2.0 %, corresponding to approximately 1 mg per application. No adverse effects were observed across all measurements, including histological assessments, indicating that HdLP is safe for topical use on excisional wounds.

Wound healing proceeds through overlapping phases: inflammation, proliferation, and remodeling**.** In the inflammatory phase, edema and hyperemia are macroscopically visible. Treatment with HdLP (2.0 %) reduced edema without affecting hyperemia. Neutrophil infiltration and NO production, indicators of oxidative stress and tissue damage, were also attenuated by HdLP, suggesting a protective effect during acute inflammation.

Macrophage activation is critical for transitioning from inflammation to proliferation, as these cells release mediators that promote angiogenesis and fibroblast proliferation [30, 31]. HdLP increased IL-1β and IL-10 levels without affecting TNF-α, suggesting modulation of macrophage activity to support tissue homeostasis. Similar observations have been reported for latex proteins from Calotropis procera, although differences in proteomes among plant latexes complicate direct comparisons [24, 32]. Unlike H. drasticus, which is orally consumed, C. procera latex has documented toxic effects.

Re-epithelialization, the initial event in the proliferative phase, involves keratinocyte proliferation and migration to restore the epidermal barrier [33]. In this study, HdLP (2.0 %) significantly increased the length of the re-epithelialized layer by day 9, indicating accelerated keratinocyte proliferation compared to controls.

The proliferative phase of wound healing is driven by the coordinated activity of keratinocytes, fibroblasts, and endothelial cells, involving extracellular matrix production and revascularization**.** Beyond restoring the skin's physical barrier, keratinocytes play a regulatory role over fibroblasts by secreting growth factors and enzymes that contribute to tissue remodeling. Histological analyses allow evaluation of these cellular events. In the current study, fibroblast proliferation was higher on day 14 in wounds treated with 2.0 % HdLP compared to the vehicle group, indicating that topical HdLP enhances the proliferative phase of healing.

As inflammation subsides, tissue remodeling begins, marking the final stage of the healing process**.** This phase focuses on generating and organizing functional tissue until full maturation is achieved. Remodeling commences in the late proliferative phase and involves the synthesis of type III collagen, which is later replaced by type I collagen as part of structural maturation. The provisional extracellular matrix, containing fibroblasts, myofibroblasts, and macrophages, is initially composed of immature collagen (type III), proteoglycans, glycosaminoglycans, fibronectin, and hyaluronic acid [34]. Collagen synthesis is therefore critical for rebuilding the extracellular matrix and restoring tissue integrity. The increased fibroblast proliferation observed aligns with the higher collagen deposition seen in remodeling tissue treated with 2.0 % HdLP on day 14 (**Figure 6b**). HdLP also enhanced tissue organization, reduced cellularity, and promoted keratinization of the epithelium at this stage **(Figure 7d)**, suggesting an overall improvement in the healing process. Similar effects have been reported for latex proteases from Wrightia tinctoria and Plumeria rubra, which increased collagen deposition and accelerated wound contraction [35, 36].

HdLP represents the soluble protein fraction of H. drasticus latex, accounting for 3.1 % of the freeze-dried latex. Folk medicine practices using diluted latex in water provide approximately half of this protein content. The present study focused on these proteins because latex proteins from other species, such as Calotropis procera, have been shown to enhance wound healing [22, 24, 25]. Our results indicate that HdLP similarly promotes efficient tissue repair. While both H. drasticus and C. procera latex proteins share this healing property, their proteomes are distinct. C. procera latex contains multiple protein isoforms, including peptidases, chitinases, and antioxidant enzymes, whereas H. drasticus latex has a much simpler protein profile, with osmotin being the only confirmed common protein. Current knowledge regarding latex proteins is limited, including their constitutive nature, seasonal variations, or other specificities. Cho *et al*. [37] identified a set of common proteins across different plant latexes, suggesting functional links to synthesis, plant defense, and ecological roles. Notably, H. drasticus latex is comparatively poor in protein content, highlighting the need for further characterization of HdLP.

Gastric ulcers involve tissue damage and inflammation, which are key features also observed in the excisional wound model used in this study to evaluate HdLP. However, the wound healing effects observed here cannot be directly extrapolated to gastric ulcers. Testing HdLP for gastric ulcer therapy would require oral administration in an appropriate ulcer model, which differs significantly from the topical approach employed in the current study.

An osmotin protein, also found in C. procera latex, was detected in H. drasticus latex [38]. Osmotins are common plant defense proteins and appear to be widely conserved across latex-producing species [2]. These proteins have been reported to mimic the human hormone adiponectin and induce pro-inflammatory effects by promoting IL-1β release [39]. This aligns with our finding that HdLP increased IL-1β levels during the early inflammatory phase, suggesting a potential mechanism and providing a basis for further investigations into HdLP's therapeutic properties.

The absence of toxicity or adverse reactions in both in vitro and in vivo models in this study is consistent with ethnopharmacological knowledge, as local users of Janaguba latex reportedly consume it safely. Scientifically validating traditional medicinal plants is crucial not only to confirm their therapeutic value but also to encourage the protection and rational use of ethnopharmacological resources.

At the end of the healing period, HdLP-treated wounds did not show a significant difference in contraction compared to other treatment groups. This may reflect the rapid wound closure inherent to healthy mice, which lack complicating conditions that could slow healing. This highlights a limitation of the present study, as HdLP was evaluated in an acute, non-infected wound model. Future studies should explore whether HdLP is equally effective in chronic or complicated wounds, such as those associated with diabetes or venous insufficiency.

Conclusion

Topical application of proteins isolated from H. drasticus latex improved the healing of excisional wounds compared to vehicle controls and a commercial product designed for wound repair. The enhanced healing was associated with modulation of the inflammatory response and more mature tissue remodeling, as indicated by histological and biochemical analyses. This study identifies wound healing as a previously unreported property of H. drasticus latex. While the findings are promising, they are limited to a single acute wound model, and further studies are needed to elucidate the in vitro mechanisms and molecular targets underlying HdLP's therapeutic effects.

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