International Journal Of Health, Engineering And Technology (IJHET) *E-ISSN 2829 - 8683*
Volume 5, Number 2, July 2026, *Page. 2293 - 2303*
Email : editorijhess@gmail.com

The Effect Of Moringa Oleifera Leaf Extract On Fibroblast Cell Migration And Morphology In In Vitro Nih/3t3 Cell Line Culture

Mawar Subangkit[1)], Made Intan Dwijayanti[2)], Elpita Tarigan[3)], Yusa Irarang[4)], Sindi Farhana[5)]
[1)] School of Veterinary Medicine and Biomedicine, IPB University, Bogor, Indonesia
[2)] Veterinary Medicine Study Program, Faculty of Medicine and Veterinary Medicine, Nusa Cendana University, Kupang, Indonesia
[3,4,5)] iRATco Laboratory Services, Bogor, Indonesia

*Corresponding Author
E-mail: msbangkit@gmail.com

Abstract

Moringa oleifera leaves are widely recognized for containing flavonoid and phenolic compounds with the potential to promote wound healing by stimulating fibroblast activity. This study aimed to evaluate the effects of Moringa oleifera leaf extract on the migration and morphological changes of NIH/3T3 fibroblast cells using an in vitro wound model. The experiment employed the scratch wound healing assay with five extract concentrations (12.5, 25, 50, 100, and 200 µg/mL) and one untreated control group (Blank). Observations were conducted on days 0, 1, and 2 using an inverted microscope. Image quantification was performed with ImageJ, and non-parametric statistical analysis (Kruskal–Wallis test) was conducted using RStudio. The evaluated parameters included cell count, cell density in the peripheral wound area, and four cell morphometric parameters (solidity, circularity, roundness, and aspect ratio). The results demonstrated a significant difference in cell count among treatment groups (p = 0.00072), with the 50 and 100 µg/mL groups showing the most consistent increase in cell number on days 1 and 2. The solidity parameter also differed significantly among treatment groups at each observation time point (p = 0.0014), whereas circularity, roundness, and aspect ratio exhibited significant differences in pairwise group comparisons despite the overall Kruskal–Wallis test not reaching statistical significance. Cell density in the peripheral wound area did not differ significantly among groups (p = 0.24). The 200 µg/mL concentration consistently showed the lowest cell count and cell density on day 2, suggesting a potential cytotoxic effect at high doses. These findings support a biphasic (hormetic) dose–response pattern, with concentrations of 50–100 µg/mL providing the most consistent stimulation of fibroblast cell migration and morphological changes, closely resembling the activated healthy cell phenotype observed in the untreated control group.

Keywords: Moringa Oleifera; Fibroblas NIH/3T3; Scratch Wound Healing Assay; Cell Migration; Cell Morphometry.

INTRODUCTION

The use of medicinal plants as sources of bioactive compounds to support wound healing has long been practiced across various cultures. The *World Health Organization* reports that a large proportion of the global population continues to rely on medicinal plants for healthcare, including the treatment of acute and chronic wounds that require complex tissue regeneration. In Indonesia, data from the *Basic Health Research Survey* (*Riset Kesehatan Dasar*) indicate that the prevalence of injuries increased from 8.3% in 2013 to 9.2% in 2018, with abrasions, contusions, and lacerations being the most common types of injuries (Ministry of Health of the Republic of Indonesia, 2019). Wound healing is a dynamic process consisting of four overlapping phases: hemostasis, inflammation, proliferation, and tissue remodeling. Among these, the proliferative phase plays a crucial role, as fibroblasts actively synthesize collagen and extracellular matrix components that serve as the structural framework for new tissue formation (Li et al., 2020).

Fibroblasts are the principal cells responsible for connective tissue repair. In addition to synthesizing collagen, fibroblasts migrate toward the wound site and participate in extracellular matrix remodeling. Therefore, their proliferative and migratory capacities are key determinants of the rate of wound closure (Kusumbe & Adams, 2018). Consequently, considerable efforts have been devoted to identifying agents capable of effectively stimulating fibroblast activity, particularly natural products possessing antioxidant and anti-inflammatory properties.

Moringa oleifera, commonly known as the drumstick tree or moringa, is a member of the *Moringaceae* family that is widely cultivated in tropical and subtropical regions, including Southeast Asia, Africa, and South America (Anwar et al., 2007). Its leaves are rich in flavonoids such as quercetin and kaempferol, phenolic acids including gallic acid and chlorogenic acid, as well as vitamin C and essential minerals such as calcium, magnesium, and iron (Leone et al., 2015; Saini et al., 2016). These bioactive compounds exhibit potent antioxidant and anti-inflammatory activities that have been reported to reduce oxidative stress in wounded tissues while promoting fibroblast proliferation (Khor et al., 2016).

Recent studies have further demonstrated the wound-healing potential of *Moringa oleifera* leaf extract. Standardized aqueous leaf extract has been shown to enhance the proliferation and migration of human dermal fibroblasts and keratinocytes and has been successfully incorporated into alginate–pectin film wound dressings (Niyangoda et al., 2024). Similarly, a polyvinyl alcohol hydrogel containing *Moringa oleifera* leaf extract and graphene oxide significantly enhanced 3T3L1 fibroblast migration in a scratch assay at a concentration of 50 µg/mL compared with the control group (Ningrum et al., 2023). Another study using a diabetic rat model demonstrated that methanolic *Moringa oleifera* leaf extract accelerated the healing of infected wounds through antimicrobial and antioxidant mechanisms, accompanied by increased expression of the *VEGF* and *TGF-β1* genes, which are involved in angiogenesis and tissue proliferation (Al-Ghanayem et al., 2022). In Indonesia, topical application of *Moringa oleifera* leaf extract has also been reported to increase fibroblast numbers and collagen deposition in wound tissues, with a 15% extract concentration producing the greatest therapeutic effect compared with lower concentrations (Herdiani et al., 2022).

The effects of *Moringa oleifera* leaf extract on fibroblast activity *in vitro* are commonly evaluated using the NIH/3T3 cell line, a mouse embryonic fibroblast (*Mus musculus*) cell line first established by Todaro and Green (1963). This non-tumorigenic cell line exhibits stable growth characteristics and robust proliferative responses to various growth factors, making it a widely accepted model for pharmacological and toxicological studies (Alam et al., 2019; Bower et al., 2021). Nevertheless, NIH/3T3 cells have undergone spontaneous immortalization, which may alter several gene regulatory pathways, including those involved in cell cycle regulation and the MAPK and PI3K/AKT signaling pathways. Therefore, findings obtained using this cell line should be regarded as preliminary evidence that requires further validation using primary fibroblast models (Zhao et al., 2018).

One of the most widely used *in vitro* methods for assessing fibroblast migration is the *scratch wound healing assay* (Li et al., 2020; Kramer et al., 2013). This technique enables quantitative evaluation of wound closure, cell density around the wound margin, and changes in cell morphology during migration using image analysis software such as ImageJ. Morphometric parameters, including solidity, circularity, roundness, and aspect ratio, serve as indirect indicators of migratory activity because mechanically activated fibroblasts undergo characteristic morphological changes from a rounded shape to a more elongated spindle-shaped morphology as they migrate into the wounded area (Lee et al., 2020; Kim et al., 2021).

Based on the above considerations, this study aimed to evaluate the effects of *Moringa oleifera* leaf extract on the migration and morphological changes of fibroblasts in NIH/3T3 cell cultures using an *in vitro scratch wound healing assay*. Furthermore, the study sought to determine the concentration range that most effectively promotes fibroblast activity in the context of wound healing.

RESEARCH METHODS

Materials and Equipment

The equipment used in this study included a CO_2 incubator, Laminar Air Flow (LAF) cabinet, centrifuge, inverted microscope, Countess™ 3 FL Automated Cell Counter, micropipettes with sterile pipette tips, cell scraper, cell culture flasks, 6-well plates, 24-well plates, centrifuge tubes, sterile tube racks, and a refrigerator. The materials included *Moringa oleifera* leaf extract in capsule powder form (500 mg of pure extract per capsule), NIH/3T3 cell cultures obtained from the iRATco Laboratory with an initial viability of 100% as determined by the Countess™ cell counter, Dulbecco's Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS), Antibiotic–Antimycotic (ABM) solution, Phosphate Buffered Saline (PBS), distilled water, Trypsin–EDTA, and 70% ethanol.

Preparation of *Moringa oleifera* Leaf Extract

A total of 0.100 g of *Moringa oleifera* leaf powder was weighed using an analytical balance, transferred into a 15-mL Falcon tube, and dissolved in 10 mL of distilled water to obtain a stock concentration of 10 mg/mL. The solution was vortexed and incubated at 37°C for 1 hour to facilitate the extraction of bioactive compounds. It was then centrifuged at 3,000 rpm for 10 minutes, after which the supernatant was collected and sterilized by filtration through a 0.22-µm syringe filter. Subsequently, 200 µL of the filtered extract was mixed with 1,800 µL of complete culture medium (DMEM + FBS + ABM) to prepare a 1,000 µg/mL working stock solution. Serial dilutions were then prepared using complete medium as the diluent to obtain five treatment concentrations: 12.5, 25, 50, 100, and 200 µg/mL. Finally, 200 µL of each extract-medium solution was dispensed into individual wells of a 24-well plate according to the designated treatment groups.

Cell Subculture into Well Plates

The culture medium was prepared by mixing 1.8 mL of DMEM, 200 µL of FBS, and 20 µL of Antibiotic–Antimycotic solution for each well of two 6-well plates (12 wells in total), followed by incubation at 37°C in a humidified atmosphere containing 5% CO_2. Cells from the stock culture were washed twice with PBS to remove residual medium and serum, then detached by treatment with 150 µL of Trypsin–EDTA at 37°C for 2–5 minutes. Trypsinization was terminated by adding approximately 1 mL of complete medium, and the cell suspension was gently pipetted until homogeneous before being transferred to centrifuge tubes. The cells were centrifuged at 1,000 rpm for 5 minutes, the supernatant was discarded, and the cell pellet was resuspended in fresh culture medium as required before being seeded into the prepared well plates. The plates were subsequently incubated at 37°C with 5% CO_2 until the cells adhered to the culture surface and reached confluence.

Scratch Wound Healing Assay

Once the cells reached approximately 90–100% confluence, the culture medium was removed, and the cell monolayer was gently washed with sterile PBS to eliminate residual serum and detached cells. A straight scratch was manually created across the confluent monolayer using a sterile cell scraper, followed by another PBS wash to remove cellular debris. Fresh culture medium containing *Moringa oleifera* leaf extract at concentrations of 12.5, 25, 50, 100, and 200 µg/mL was then added to the respective treatment groups, while the untreated control (Blank) received fresh culture medium without extract supplementation. Cell migration was observed immediately after scratch creation (Day 0), as well as on Day 1 and Day 2, using an inverted microscope.

Data Analysis

Images obtained from the scratch wound healing assay at each observation time point were quantitatively analyzed using ImageJ software to determine cell count, cell density within the peripheral wound area, and four cell morphometric parameters: solidity, circularity, roundness, and aspect ratio (AR). The resulting numerical data were first tested for normality and subsequently analyzed using the non-parametric Kruskal–Wallis test in RStudio because the data did not satisfy the assumption of normal distribution across treatment groups. The results were presented as line plots illustrating temporal changes in each parameter from Day 0 to Day 2 for each treatment concentration,

together with the overall *p*-value from the Kruskal–Wallis test and significance annotations (*ns*, *, **, ***) indicating pairwise comparisons among treatment groups at each observation time point.

RESULTS AND DISCUSSION

Initial Cell Density

The following are the results of the cell count calculation:

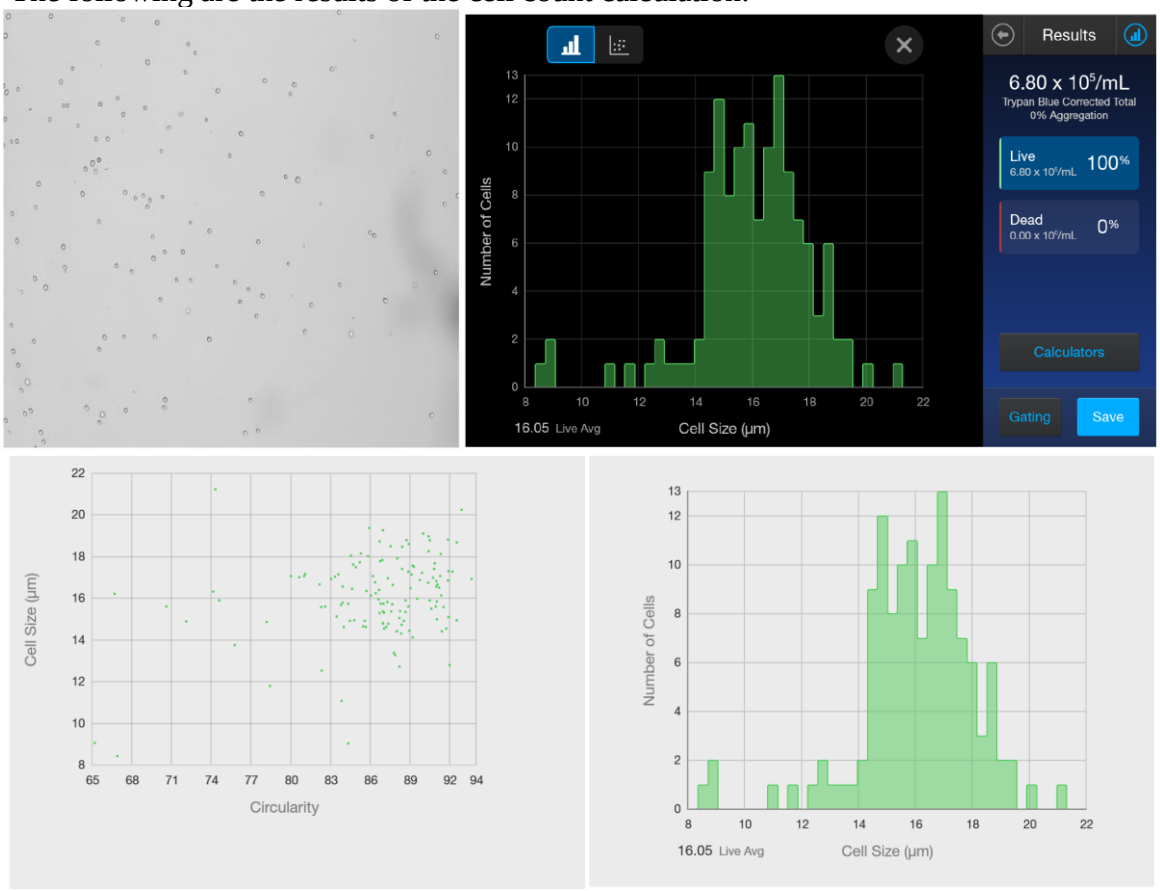

Figure 1.

Cell counting using a Countess 3FL Automated Cell Counter prior to scratch preparation showed that all treatment groups had uniform initial cell densities, reaching confluences ranging from 90–100%. This equal initial density across groups ensures that differences in cell migration observed at later stages are not influenced by inoculation cell count bias, allowing valid comparisons between treatment groups.

Cell Counts in the Migration Area

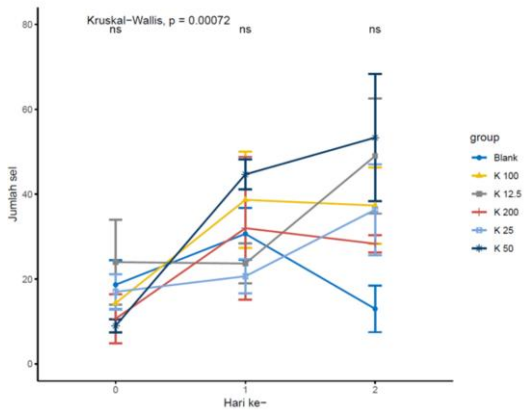

Figure 2.

Analysis of cell counts in the scratch area showed a statistically significant difference between treatment groups based on the Kruskal-Wallis test (p = 0.00072). On day 0, all groups showed relatively low initial cell counts and did not differ significantly (ranging from 8 to 24 cells). Entering day 1, all extract treatment groups (K12.5 to K200) and the control group experienced an increase in cell count, with the K50 group showing the highest value (mean approaching 44 cells), followed by K100 and K200. On day 2, the K12.5 and K50 groups showed the highest cell counts (approaching 49 and 53 cells, respectively), followed by K100 and K25, which also increased. While the Blank (control) group experienced a sharp decrease from day 1 to day 2 (from approximately 31 to 13 cells). The K200 group showed a more gradual increase in cell count compared to the medium-dose group, with values on day 2 falling below those of K12.5, K50, K100, and K25.

Cell Density in the Peripheral Wound Area

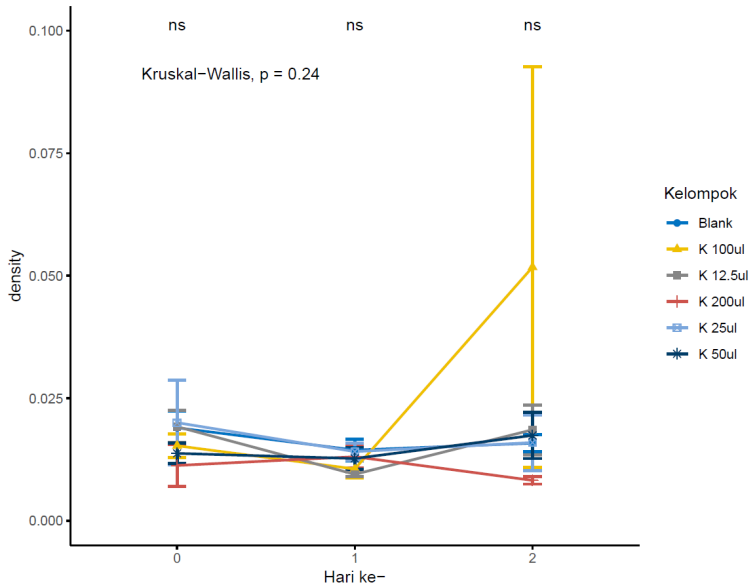

Figure 3.

In contrast to cell count parameters, analysis of cell density in the peripheral area (around the wound) showed no significant differences between groups in the overall Kruskal-Wallis test (p = 0.24), denoted by "ns" at all observation time points. On day 0, all groups showed relatively homogeneous initial densities in the range of 0.011–0.020. On day 1, density values between groups tended to be close to each other in the range of 0.010–0.016. On day 2, the K100 group showed a significant increase in density (mean above 0.05) but with a very large standard error (approaching 0.09), while the K200 group showed the lowest density among all groups on day 2 (below 0.01). The Blank, K12.5, K25, and K50 groups showed a relatively stable pattern without sharp spikes or declines throughout the observation period.

Cell Morphometric Parameters

Four cell morphometric parameters were analyzed to describe changes in fibroblast shape during the migration process.

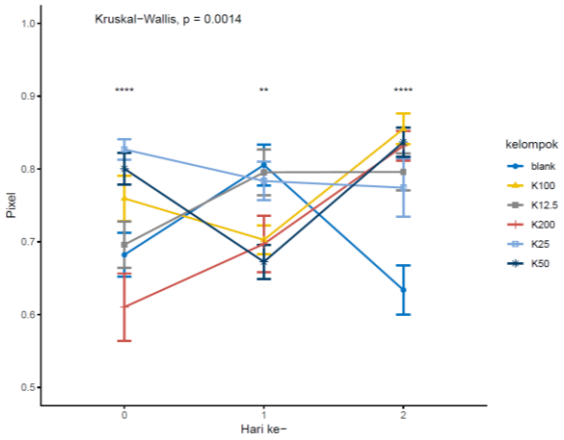

Figure 4.

Solidity showed a significant overall difference between groups (p = 0.0014), with intergroup differences also significant at each time point (day 0: p < 0.0001; day 1: p < 0.01; day 2: p < 0.0001). On day 0, the K200 group showed the lowest solidity value (≈0.61), while the K25 and K50 groups showed the highest values (≈0.82–0.83). On day 2, this pattern reversed: the K100, K50, and K200 groups showed the highest solidity values (≈0.82–0.86), while the Blank group showed the sharpest decline in solidity values (≈0.63).

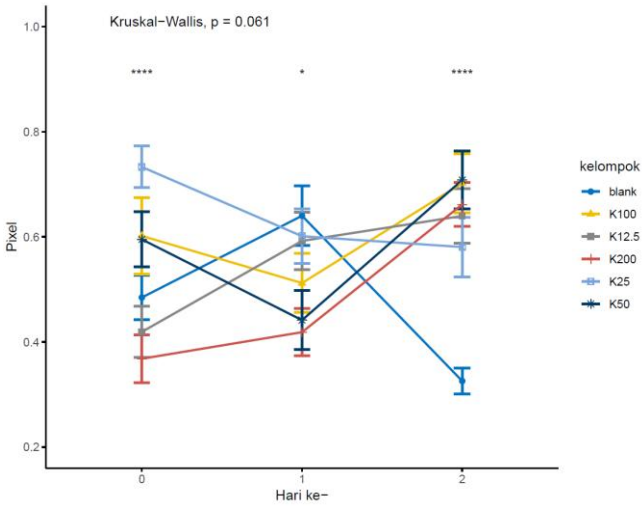

Figure 5.

Circularity showed a trend toward differences between groups (p = 0.061 in the overall test), but pairwise comparisons at each time point remained significant (day 0 and day 2: p < 0.0001; day 1: p < 0.05). The Blank group showed a unique pattern of increasing circularity values from day 0 (≈0.48) to day 1 (≈0.64), then dropping sharply on day 2 (≈0.33). The K200 group consistently showed the lowest circularity values from day 0 to day 1 (≈0.37–0.42), before increasing on day 2 (≈0.65).

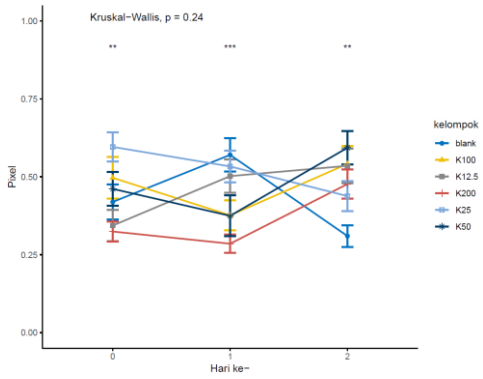

Figure 5.

Roundness was not significant overall (p = 0.24) but was significant in the intergroup comparison at each time point (day 0: p < 0.01; day 1: p < 0.001; day 2: p < 0.01). The K200 group consistently demonstrated the lowest roundness values on days 0 and 1 (≈0.28–0.33), while the Blank group demonstrated the highest roundness values on day 1 (≈0.57) before declining again on day 2 (≈0.31).

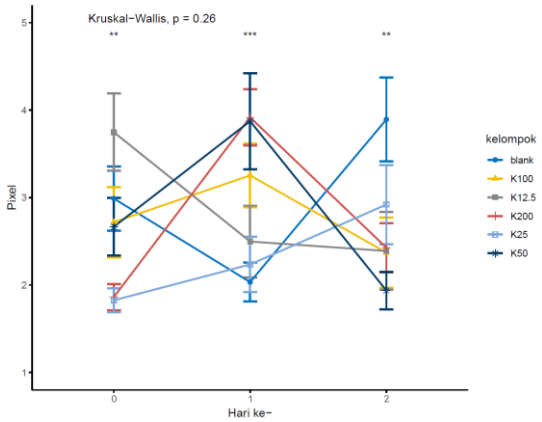

Figure 6.

Aspect ratio (AR) was also not significant overall (p = 0.26) but was significant in the intergroup comparison at each time point (day 0: p < 0.01; day 1: p < 0.001; day 2: p < 0.01). The AR value for the Blank group increased sharply from day 1 (≈2.0) to day 2 (≈3.9), in line with a decrease in circularity and roundness values in the same group, which consistently indicates cell elongation. The K200 group showed the highest AR value on day 1 (≈3.9) before decreasing again on day 2 (≈2.4).

Discussion

Uniform initial cell density across treatment groups is an important prerequisite for valid comparisons of cell migration in subsequent stages. With equivalent initial densities, variations in cell number, density, and morphology observed on days 1 and 2 can be attributed to differences in biological responses to the extract treatment, not to technical bias due to differences in the number of cells seeded.

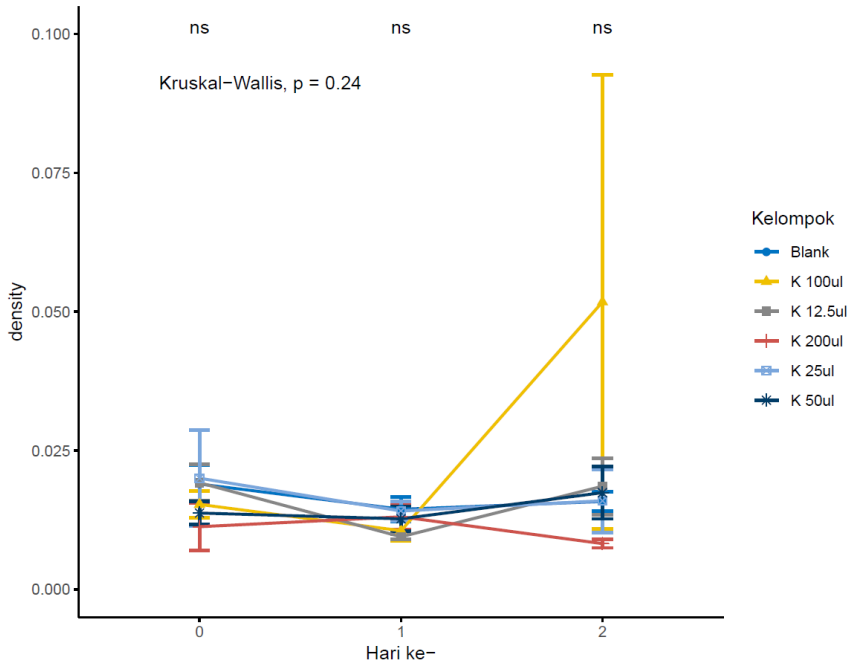

Figure 7.

The finding that the number of cells within the migration area differed significantly among treatment groups (p = 0.00072) provides strong evidence that *Moringa oleifera* leaf extract influences the dynamics of cell repopulation in the artificial wound area. The observed pattern demonstrated that the intermediate-dose groups, particularly K50, consistently exhibited the highest cell counts on Day 1 and maintained high cell numbers on Day 2, whereas the control group (Blank) showed a marked decline on Day 2. The reduction observed in the Blank group was most likely not attributable to cell death but rather to the absence of exogenous stimulation, resulting in a slower rate of cell repopulation compared with groups receiving bioactive compounds from the extract. The increased cell numbers observed in the K50 and K100 groups are consistent with reports indicating that flavonoids and phenolic acids present in *Moringa oleifera* leaves enhance the production of growth factors such as transforming growth factor-beta (TGF-β) and vascular endothelial growth factor (VEGF), both of which promote cell repopulation during wound healing (Al-Ghanayem et al., 2022). These findings are also in agreement with previous studies using *Moringa*-based hydrogel formulations, which demonstrated enhanced migration of 3T3 fibroblasts at a concentration of 50 µg/mL (Ningrum et al., 2023).

In contrast to the cell count parameter, cell density in the peripheral wound area did not differ significantly among treatment groups (p = 0.24). The discrepancy between the significant differences observed in cell counts and the non-significant differences in cell density may be explained by the distinct measurement regions. Cell counts were quantified within the scratch area (the wound center), where active cell migration primarily occurs, whereas cell density was measured in the peripheral region surrounding the wound. This pattern suggests that the stimulatory effect of *Moringa oleifera* leaf extract was more pronounced in promoting direct repopulation of the wound area than in influencing cell redistribution in the surrounding regions. Moreover, substantial variability among replicates in several treatment groups, particularly K100 on Day 2, which exhibited a large standard error, likely reduced the statistical sensitivity for this parameter, a common limitation in experimental designs with relatively few biological replicates.

The morphometric parameters provided complementary evidence supporting the quantitative findings on cell number and density. Solidity exhibited significant differences both overall and at each observation time point, with the Blank group showing the greatest decline in solidity on Day 2, whereas the intermediate- and high-dose groups (K50, K100, and K200) maintained relatively high solidity values. Similar trends were observed for circularity, roundness, and aspect ratio. The Blank group displayed a characteristic pattern of morphological change characterized by increased cell elongation, reflected by decreased circularity and roundness and an increased aspect ratio from Day 1 to Day 2. This transition from a rounded to a spindle-shaped morphology represents a well-established morphological hallmark of activated fibroblasts migrating toward the wound area, as reported in ImageJ-based fibroblast morphometric analyses using circularity and aspect ratio parameters (Lee et al., 2020; Kim et al., 2021). Therefore, the Blank group in the present study served as a biological reference illustrating the natural response of healthy fibroblasts to mechanical scratch stimulation in the absence of exogenous compounds. The delayed elongation pattern observed in this group, becoming clearly evident only on Day 2, corresponded with its lower cell count at the same time point.

Within the extract-treated groups, morphometric analysis on Day 0 revealed that K200 exhibited the lowest solidity, circularity, and roundness values from the beginning of the observation period, followed by an increase in these parameters on Day 2. This apparent reversal may indicate that, at the highest concentration, cells initially displayed an elongated morphology, possibly due to cellular stress, before subsequently experiencing reduced migration or morphological retraction during later observation periods. This interpretation is consistent with the concurrent decline in cell number and density observed in the same treatment group. Such findings align with the principle of hormesis in plant-derived bioactive compounds, which describes a biphasic dose-response characterized by stimulatory effects at low-to-moderate concentrations and inhibitory or mildly cytotoxic effects at higher concentrations (Calabrese & Kozumbo, 2021; Calabrese et al., 2022). Similar responses have

been reported for flavonoids and plant phenolic compounds, in which excessive concentrations increase the production of reactive oxygen species (ROS). At low concentrations, ROS function as signaling molecules that promote proliferation, whereas at high concentrations they induce oxidative stress and disrupt cellular membrane integrity (Chirumbolo, 2011).

Overall, the integration of cell count, cell density, and morphometric data indicates that concentrations ranging from 50 to 100 µg/mL produced the most favorable biological response. These concentrations consistently increased the number of migrating cells while inducing morphological changes characteristic of active fibroblast migration, without exhibiting the reductions in cell number or density observed at the highest concentration (200 µg/mL). This response pattern further supports the bell-shaped dose-response curve commonly reported in studies evaluating the biological activity of plant extracts on fibroblasts (Calabrese et al., 2022), suggesting that intermediate concentrations may represent the optimal therapeutic range for further development of *Moringa oleifera* leaf extract as a wound-healing agent.

Nevertheless, several limitations should be acknowledged. Some parameters, particularly peripheral cell density and the overall *p*-values for circularity, roundness, and aspect ratio, did not consistently achieve statistical significance. This was likely due to the limited number of biological replicates included in this mini-project study, which reduced the sensitivity of the non-parametric Kruskal–Wallis test for detecting differences among treatment groups, especially for parameters exhibiting substantial inter-replicate variability. Furthermore, the use of the spontaneously immortalized NIH/3T3 cell line should be considered when interpreting these findings, as this cell line is known to exhibit alterations in gene regulation associated with cell cycle progression and the MAPK/PI3K-AKT signaling pathways, potentially limiting its ability to fully represent the biological behavior of primary fibroblasts (Zhao et al., 2018). Therefore, future studies incorporating larger sample sizes, independent post-termination cell counting methods, and additional viability assays such as MTT or LDH assays at higher extract concentrations are warranted to strengthen the evidence regarding the cytotoxic threshold of *Moringa oleifera* leaf extract in fibroblast cells.

CONCLUSIONS

Moringa oleifera leaf extract influenced the migration dynamics of NIH/3T3 fibroblast cells in an in vitro scratch wound healing assay, as demonstrated by significant differences in cell numbers among treatment groups ($p = 0.00072$) and significant changes in cell morphometric parameters at each observation time point, particularly solidity ($p = 0.0014$). The 50 and 100 µg/mL concentrations consistently produced the greatest increase in cell numbers on Days 1 and 2 compared with the control group and the other treatment groups, accompanied by morphological changes indicative of active fibroblast migration. In contrast, the 200 µg/mL concentration exhibited a declining trend in both cell number and cell density on Day 2, suggesting a possible inhibitory or mildly cytotoxic effect at higher doses. Cell density in the peripheral wound area did not differ significantly among treatment groups, indicating that the stimulatory effect of the extract was more pronounced in promoting repopulation within the central wound area than in the surrounding peripheral region. Overall, these findings support the existence of a biphasic dose-response pattern of *Moringa oleifera* leaf extract on fibroblast activity, with intermediate concentrations (50–100 µg/mL) representing the most promising range for further development as a natural wound-healing therapeutic agent.

REFERENCES

Al-Ghanayem, A. A., Alhussaini, M. S., Asad, M., & Joseph, B. (2022). Moringa oleifera leaf extract promotes healing of infected wounds in diabetic rats: Evidence of antimicrobial, antioxidant and proliferative properties. *Pharmaceuticals*, 15(5), 528. https://doi.org/10.3390/ph15050528

Alam, S., Kaur, G., & Tripathi, R. (2019). NIH 3T3 cell line as a reliable in vitro model for screening of drugs and evaluation of cytotoxicity. *Toxicology Reports*, 6, 1213–1220. https://doi.org/10.1016/j.toxrep.2019.11.002

Anwar, F., Latif, S., Ashraf, M., & Gilani, A. H. (2007). Moringa oleifera: A food plant with multiple medicinal uses. *Phytotherapy Research*, 21(1), 17–25. https://doi.org/10.1002/ptr.2023

Bower, J. J., Karunasena, E., & Mayo, L. D. (2021). Using NIH 3T3 cells to identify transforming potential of oncogenes. *Methods in Molecular Biology*, 2221, 75–88. https://doi.org/10.1007/978-1-0716-0997-1_6

Calabrese, E. J., & Kozumbo, W. J. (2021). The phytoprotective agent sulforaphane prevents inflammatory degenerative diseases and age-related pathologies via Nrf2-mediated hormesis. *Pharmacological Research*, 163, 105283. https://doi.org/10.1016/j.phrs.2020.105283

Calabrese, E. J., Dhawan, G., Kapoor, R., Agathokleous, E., & Calabrese, V. (2022). Hormesis: Wound healing and fibroblasts. *Pharmacological Research*, 184, 106449. https://doi.org/10.1016/j.phrs.2022.106449

Chirumbolo, S. (2011). Hormesis, resveratrol and plant-derived polyphenols: Some comments. *Human and Experimental Toxicology*, 30(8), 1027–1031.

Herdiani, M., Pramasari, C. N., & Purnamasari, C. B. (2022). Pengaruh ekstrak daun kelor (Moringa oleifera Lam.) terhadap penyembuhan luka. *Mulawarman Dental Journal*, 2(1), 16–29. https://doi.org/10.30872/MOLAR.v2i1.5533

Kementerian Kesehatan RI. (2019). *Laporan Nasional Riset Kesehatan Dasar (Riskesdas) 2018*. Badan Penelitian dan Pengembangan Kesehatan, Kementerian Kesehatan RI.

Khor, K. Z., Lim, V., Moses, E. J., Lai, C. S., & Yusoff, K. (2016). Wound healing potential of Moringa oleifera flower and leaf extracts on fibroblast cell migration and angiogenesis. *Journal of Ethnopharmacology*, 188, 204–212. https://doi.org/10.1016/j.jep.2016.04.047

Kim, H. J., Park, S. Y., & Lee, J. Y. (2021). Wound healing potential of plant-derived compounds evaluated using NIH 3T3 fibroblast model. *Journal of Ethnopharmacology*, 274, 113995. https://doi.org/10.1016/j.jep.2021.113995

Kramer, N., Walzl, A., Unger, C., Rosner, M., Krupitza, G., Hengstschläger, M., & Dolznig, H. (2013). In vitro cell migration and invasion assays. *Mutation Research/Reviews in Mutation Research*, 752(1), 10–24. https://doi.org/10.1016/j.mrrev.2012.08.001

Kusumbe, A. P., & Adams, R. H. (2018). Fibroblasts in tissue repair and disease. *Cell*, 172(3), 314–329. https://doi.org/10.1016/j.cell.2018.01.002

Lee, M., Jeong, S. Y., & Lim, H. J. (2020). Evaluation of wound healing effects of natural products using NIH 3T3 fibroblast migration assay. *Pharmaceutical Biology*, 58(1), 937–946. https://doi.org/10.1080/13880209.2020.1807155

Leone, A., Spada, A., Battezzati, A., Schiraldi, A., Aristil, J., & Bertoli, S. (2015). Moringa oleifera seeds and oil: Characteristics and uses for human health. *International Journal of Molecular Sciences*, 16(12), 12791–12835. https://doi.org/10.3390/ijms160612791

Li, J., Chen, J., & Kirsner, R. (2020). Pathophysiology of acute wound healing. *Clinics in Dermatology*, 38(5), 563–570. https://doi.org/10.1016/j.clindermatol.2020.04.004

Ningrum, D. R., Hanif, W., Mardhian, D. F., & Asri, L. A. T. W. (2023). In vitro biocompatibility of hydrogel polyvinyl alcohol/Moringa oleifera leaf extract/graphene oxide for wound dressing. *Polymers*, 15(2), 468. https://doi.org/10.3390/polym15020468

Niyangoda, D., Muayad, M., Tesfaye, W., Bushell, M., Ahmad, D., Samarawickrema, I., Sinclair, J., Kebriti, S., Maida, V., & Thomas, J. (2024). Development and formulation of Moringa oleifera

standardised leaf extract film dressing for wound healing application. *Frontiers in Pharmacology*, 15, 1288382. https://doi.org/10.3389/fphar.2024.1288382

Saini, R. K., Sivanesan, I., & Keum, Y. S. (2016). Phytochemicals of Moringa oleifera: A review of their nutritional, therapeutic and industrial significance. *3 Biotech*, 6(2), 203. https://doi.org/10.1007/s13205-016-0526-3

Todaro, G. J., & Green, H. (1963). Quantitative studies of the growth of mouse embryo cells in culture and their development into established lines. *The Journal of Cell Biology*, 17(2), 299–313. https://doi.org/10.1083/jcb.17.2.299

Zhao, Y., Li, Y., & Wang, X. (2018). Gene expression profile of NIH 3T3 cells compared to primary fibroblasts: Implications for experimental design. *Molecular Genetics and Genomics*, 293(4), 949–959. https://doi.org/10.1007/s00438-018-1445-4