BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 14, Number 2, October 2025 | Pages: 951-955 | DOI: 10.14421/biomedich.2025.142.951-955 ISSN 2540-9328 (online)

Evaluation of Forest Honey on the Proliferation and Migration of Dermal Fibroblasts under Hyperglycemic Conditions: An In Vitro Study

Januar Rizqi[1]*, Denny Agustiningsih[2], Dwi Aris Agung Nugrahaningsih[3]
[1]Departement of Nursing, Faculty of Medicine, Universitas Respati Yogyakarta, Indonesia.
[2]Department of Physiology, Faculty of Medicine, Universitas Gadjah Mada, Indonesia.
[3]Department of Pharmacology and Therapy, Faculty of Medicine, Universitas Gadjah Mada, Indonesia.

Corresponding author*
arizqi.januar@respati.ac.id

Manuscript received: 01 September, 2025. Revision accepted: 27 October, 2025. Published: 28 October, 2025.

Abstract

This study aims to evaluate the effect of forest honey on fibroblast proliferation and migration under hyperglycemic conditions in vitro. The research method involved culturing primary fibroblasts in high-glucose DMEM (25 mM) and dividing them into five groups: standard control (standard medium), hyperglycemia control (high-glucose medium without treatment), 3% honey, 1.5% honey, and 0.75% honey. Proliferation was assessed by counting live cells (Trypan Blue staining/hemocytometer) at 24, 48, and 72 hours. Migration was measured using a scratch assay (0, 24, 48, 72 hours) and analyzed with ImageJ. The Shapiro–Wilk test was used for normality; data were analyzed with ANOVA followed by LSD or Kruskal–Wallis ($p<0.05$). Results showed that hyperglycemia decreased proliferation compared to the standard control. Treatment with 1.5% honey and 0.75% honey consistently increased proliferation compared to the hyperglycemic control at all time points ($p<0.05$), while 3% honey showed a relatively lower increase. The standard control group achieved 100% closure at 72 hours in the migration variable. The honey groups (0.75–3%) showed an increase compared to the hyperglycemic control, but the difference was not significant at 24–48 hours and approached significance at 72 hours ($p\approx0.057$). In conclusion, forest honey at a concentration of 1.5% can increase fibroblast proliferation in a hyperglycemic environment. The effect on migration requires confirmation with osmolality controls and more sensitive endpoints. These findings provide a biological basis for developing honey-based diabetic wound care adjuvants focusing on dose optimization and formulation standardization.

Keywords: forest honey; in vitro hyperglycemia (25 mM); dermal fibroblast proliferation; scratch migration assay.

INTRODUCTION

Diabetes mellitus (DM) continues to increase and has become one of the most significant burdens on the global health system, with a significant economic impact on individuals (IDF, 2025). One of the complications of DM is diabetic foot ulcer (DFU), which can lead to disability, amputation, and mortality, and requires high healthcare resources (Fuentes-Peñaranda et al., 2025). Wound healing is a coordinated process that includes the phases of hemostasis, inflammation, proliferation, and remodeling, with complex intercellular orchestration and mediators, one of which is the fibroblast (Mamun et al., 2024).

Fibroblasts play a central role in matrix deposition, wound contraction, and the secretion of growth factors that support angiogenesis and tissue repair (Boraldi et al., 2024). Reactive oxygen species (ROS) support healing signals under controlled conditions, while in excessive conditions, they promote chronicity of wounds (Hunt et al., 2024). Redox imbalance disrupts proliferation and migration by disrupting the cytoskeleton, adhesion–de-adhesion, and signal transduction. Hyperglycemia exacerbates the condition through the AGE–RAGE pathway, mitochondrial load, and persistent inflammation, thereby delaying the proliferation phase (Hunt et al., 2024). In human dermal fibroblasts, high glucose exposure is associated with cellular aging and dysfunction that impedes healing (Ma et al., 2023). Therefore, restoring redox homeostasis and fibroblast function is a biological key to accelerating tissue repair (Mamun et al., 2024).

Hyperglycemia consistently reduces fibroblast proliferation and migration. Impeded cell-type-specific migration is recognized as a key mechanism delaying diabetic wound healing (Song et al., 2025). Current wound therapies such as nanotechnology, stem cells, and innovative dressings are promising, but cost and complexity constraints limit their widespread application (Mamun et al., 2024). Some high-glucose studies have not included iso-osmotic controls, making it challenging to distinguish biological effects from osmolality or viscosity (Sharma et al., 2018). From a methodological perspective, further evidence is needed to strengthen in vitro evidence and enable translation (Mamun et al.,

2024). Thus, scientific, biocompatible adjuvant interventions that can be integrated into wound care practices are a fundamental need (Fuentes-Peñaranda et al., 2025).

Honey is a candidate adjuvant due to its polyphenol or flavonoid content and its ability to produce low doses of H_2O_2 via glucose oxidase, making it relevant as a redox modulator and antimicrobial (Tashkandi, 2021). Honey can also modulate inflammation, support the proliferation phase, increase collagen synthesis, and improve cell migration (Iosageanu et al., 2024). Previous studies have assessed the effectiveness of honey on various chronic wounds with a good safety profile, although the heterogeneity of studies calls for further standardization. Variability in sources and phenolic composition underscores the importance of precise dosing to ensure efficacy without causing osmotic stress (Saad, 2025). Therefore, conducting safe concentration range trials for fibroblasts under hyperglycemic conditions is crucial.

Although chronic wound therapy rapidly evolves to include nanotherapeutics, exosomes, and innovative dressings, safe, affordable, and accessible adjuvants remain highly needed (Mamun et al., 2024). A significant gap in this research is the limited number of studies evaluating honey on fibroblast proliferation and migration under hyperglycemic conditions. The research of this article lies in its focus on forest honey across a range of doses and the simultaneous evaluation of two key fibroblast functions in a hyperglycemic environment. Thus, this study aimed to assess the effect of forest honey on fibroblast proliferation and migration under hyperglycemic conditions in vitro.

MATERIALS AND METHODS

Study Area

This study was conducted at the Pharmacology Laboratory, Physiology Laboratory, and Anatomy Laboratory of the Faculty of Medicine, Gadjah Mada University, Yogyakarta, and has obtained ethical approval from the UGM LPPT Research Ethics Committee (No. 00010/04/LPPT/2016).

Cell Culture

Primary fibroblasts were obtained from the dermal tissue of male Wistar strain white rats (aged 3–4 months) induced with diabetes mellitus using streptozotocin (STZ, 60 mg/kg BW) and nicotinamide (NA, 120 mg/kg BW) via intraperitoneal injection. Mice with fasting blood glucose levels ≥126 mg/dL were categorized as successful diabetes models. Fibroblast cells were isolated enzymatically, then cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS) and antibiotics-antimycotics. The cultures were maintained in a 5% CO_2 incubator at 37 °C until they reached 80–90% confluence. Hyperglycemia was modeled using high-glucose DMEM medium (25 mM).

Experimental Groups and Treatments

The fibroblast cultures were divided into five groups, namely: (1) fibroblasts in standard medium (standard control), (2) fibroblasts in high-glucose medium without treatment (hyperglycemia control), (3) 3% honey fibroblasts, (4) 1.5% honey, and (5) 0.75% honey. Each treatment was performed in triplicate (n = 3) and observed after 24, 48, and 72 hours of incubation.

Proliferation Assay

Cell proliferation was assessed by counting the number of cells using a hemocytometer after Trypan Blue staining. The number of live cells was counted 24-, 48-, and 72-hours post-treatment, then expressed as the mean ± standard deviation.

Migration Assay

Fibroblast migration activity was tested using the scratch assay method. A linear scratch was made using a sterile pipette tip in confluent monolayer cultures. The scratch area was photographed with an inverted microscope at 0, 24, 48, and 72 hours. Wound closure was calculated using ImageJ software, and the results were expressed as the percentage of cell migration relative to the baseline.

Statistical Analysis

Data normality were tested using the Shapiro–Wilk test. Normally distributed data were analyzed using One-Way ANOVA followed by LSD post hoc testing. The Kruskal–Wallis's test was used if the data were not normally distributed. The significance level was set at $p < 0.05$.

RESULTS AND DISCUSSION

Fibroblast Proliferation

Fibroblast proliferation differed between groups at all observation time points (24, 48, 72 hours). Standard control (standard medium) showed the highest number of cells, while hyperglycemia control (high glucose medium without treatment) showed the lowest. Administration of 1.5% honey and 0.75% honey consistently increased proliferation compared to the hyperglycemia control at 24, 48, and 72 hours (all $p<0.05$; LSD post hoc test), while 3% honey increased but was relatively lower than 1.5% and 0.75% honey. The Shapiro–Wilk test met the normality assumption, and One-Way ANOVA showed significant differences between groups ($p<0.001$). The mean and standard deviation for each group is shown in Table 1.

Table 1. Mean number of fibroblasts (mean ± SD) at 24, 48, and 72 hours.

Group	24 h (mean ± SD)	48 h (mean ± SD)	72 h (mean ± SD)
Standard control	40.68 ± 4.62	49.32 ± 4.46	51.63 ± 1.36
Hyperglycemic control	16.82 ± 2.86	21.14 ± 4.36	19.19 ± 3.86
Honey 3%	23.51 ± 2.47	30.06 ± 7.55	22.29 ± 3.54
Honey 1.5%	30.08 ± 2.70	36.01 ± 5.87	29.33 ± 2.65
Honey 0.75%	28.61 ± 1.71	31.18 ± 3.90	27.79 ± 1.75

Note: ANOVA p<0.001; LSD follow-up test shows that 1.5% honey (24 hours p=0.019; 48 hours p=0.011; 72 hours p=0.006) and 0.75% honey (24 hours p=0.035; 48 hours p=0.046; 72 hours p=0.012) were higher than the hyperglycemia control.

Fibroblast Migration

Migration activity increased over time in all groups. The standard control showed the highest migration percentage and reached 100% closure at 72 hours, while the hyperglycemia control was the lowest. Treatment with 3% honey, 1.5% honey, and 0.75% honey showed an increasing trend compared to the hyperglycemia control; the differences between groups were not significant at 24 hours (Kruskal–Wallis p=0.629) and 48 hours (p=0.094), and showed a trend at 72 hours (ANOVA p=0.057). Detailed values are presented in Table 2.

Table 2. Percentage of fibroblast migration in various treatment groups.

Group	24 h (median; IQR)	48 h (median; IQR)	72 h (mean ± SD)
Standard control	78.93 (69.73–79.02)	100.00 (100.00) [a]	100.00 ± 0.00[a]
Hyperglycemic control	78.59 (66.50–78.64)	81.23 (68.84–82.29)	79.23 ± 7.69
Honey 3%	78.40 (65.02–78.47)	82.70 (68.66–83.65)	79.92 ± 8.21
Honey 1.5%	77.07 (66.02–77.07)	83.16 (70.12–85.38)	81.20 ± 7.45
Honey 0.75%	77.79 (65.18–79.34)	81.71 (68.93–82.44)	80.35 ± 7.61

[a]The difference is significant compared to other groups (p<0.05).

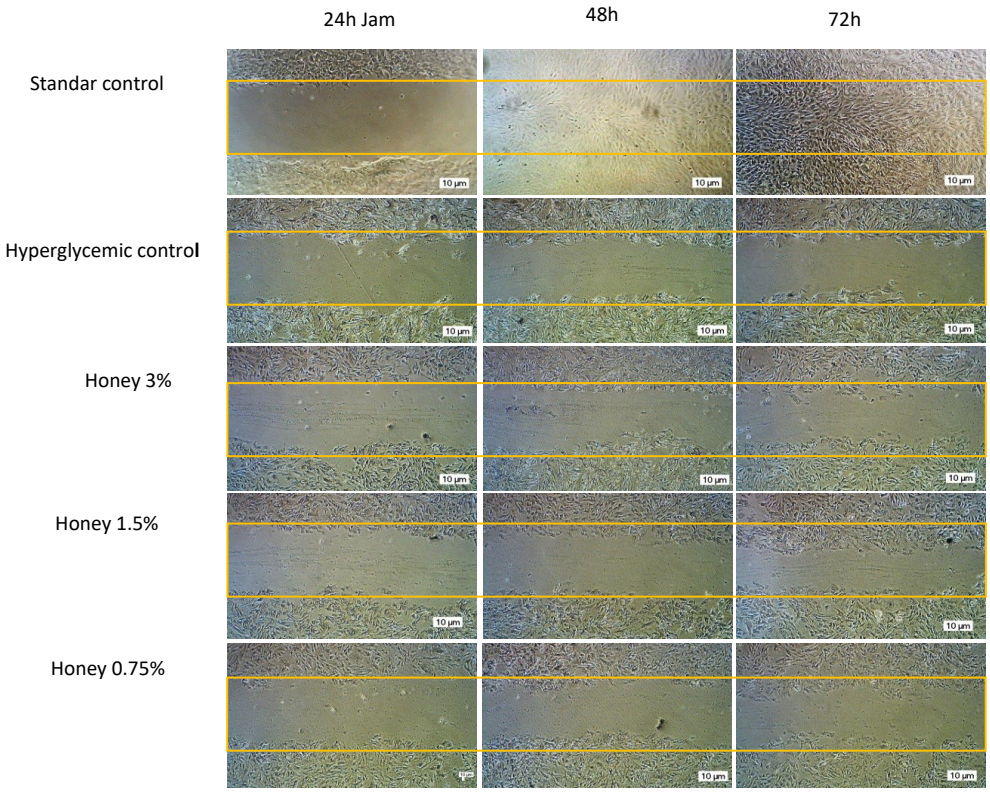

Figure 1. Fibroblast migration activity at 24, 48, and 72 hours (inverted microscope 100×). Straight lines mark the initial scratch boundaries. Groups: standard control, hyperglycemia control, 3% honey, 1.5% honey, 0.75% honey.

Discussion

This study shows that hyperglycemia suppresses fibroblast function, particularly proliferation, compared to normoglycemic controls; conversely, honey at 0.75–1.5% consistently increased proliferation compared to hyperglycemic controls, while honey at 3% did not increase proliferation. Meanwhile, fibroblast migration in the honey group showed an increasing trend compared to the hyperglycemic control, but did not reach statistical significance; the normoglycemic control achieved 100% wound closure at 72 hours. These results are consistent with the knowledge that a high-glucose environment disrupts redox homeostasis and tissue repair dynamics, while the bioactive components of honey have the potential to moderate these disturbances (Berry et al., 2024; Pasupuleti et al., 2020).

The finding of reduced fibroblast performance in high-glucose media is consistent with reports that hyperglycemia increases oxidative stress and maintains inflammation, thereby delaying the proliferation phase until remodeling (González et al., 2023; Nagy et al., 2019). In human dermal cell models, high glucose exposure is associated with cellular aging and functional abnormalities that slow healing (Zhang et al., 2021). Research results show that honey, especially honey rich in phenolics, can suppress inflammation, support proliferation, and improve cell migration.

Hyperglycemia increases ROS production through the AGE–RAGE pathway, mitochondrial load, and glucose autooxidation; excessive ROS exposure disrupts cytoskeletal organization, adhesion–de-adhesion, and signaling required for proliferation and migration (Comino-Sanz et al., 2021; Hunt et al., 2024). Honey contains polyphenols or flavonoids that produce low levels of H_2O_2 via glucose oxidase; both factors contribute to redox buffering (antiradical, pro-inflammatory resolution) and support cell cycle and matrix survival (Iosageanu et al., 2024; Wilczyńska & Żak, 2024). The dose-response correlation indicates that honey at 0.75–1.5% is more effective than honey at 3%.

Scientifically, these results reinforce the hypothesis that honey can maintain and enhance fibroblast proliferation in a hyperglycemic environment, which is a key mechanism for initiating the tissue repair phase (Pasupuleti et al., 2020; Scepankova et al., 2021). To strengthen the evidence, further studies need to measure and control osmolality, add orthogonal endpoints, and verify the redox pathway through quantification of ROS and antioxidant enzymes (SOD, CAT, GPx) (Gayathri et al., 2023). Testing in 3D/explant models and in vivo validation in diabetic wounds will increase biological relevance toward early clinical trials (Mamun et al., 2024).

CONCLUSIONS

Hyperglycemia reduces fibroblast function, most notably in proliferation, compared to normoglycemic controls. Administration of forest honey at concentrations of 0.75–1.5% consistently increased fibroblast proliferation compared to hyperglycemic controls at 24, 48, and 72 hours, while 3% was relatively less optimal. In migration, the honey group showed an increasing trend compared to the hyperglycemic control, but it was not statistically significant; the standard control achieved 100% wound closure at 72 hours. Overall, these findings confirm that sub-osmotic honey concentrations (≤1.5%) are more conducive to supporting fibroblast function in a hyperglycemic environment, consistent with the study objective of evaluating the effects of honey on fibroblast proliferation and migration.

Authors' Contributions: Januar rizqi conceptualization; methodology; investigation; data curation; formal analysis; visualization; writing original draft. Denny Agustiningsih provides validation, supervision, resources, writing, review & editing, and project administration. Dwi Aris' supervision; writing, review and editing. All authors contributed to interpreting the results, reviewed and approved the final manuscript for publication, and agreed to be accountable for all aspects of the work.

Competing Interests: The authors declare that there are no competing interests.

Funding: The authors declare funding if any funding.

REFERENCES

Berry, C. E., Brenac, C., Gonzalez, C. E., Kendig, C. B., Le, T., An, N., & Griffin, M. F. (2024). Natural Compounds and Biomimetic Engineering to Influence Fibroblast Behavior in Wound Healing. *International Journal of Molecular Sciences*, *25*(6). https://doi.org/10.3390/ijms25063274

Boraldi, F., Lofaro, F. D., Bonacorsi, S., Mazzilli, A., Garcia-Fernandez, M., & Quaglino, D. (2024). The Role of Fibroblasts in Skin Homeostasis and Repair. *Biomedicines*, *12*(7), 1586. https://doi.org/10.3390/biomedicines12071586

Comino-Sanz, I. M., López-Franco, M. D., Castro, B., & Pancorbo-Hidalgo, P. L. (2021). The role of antioxidants on wound healing: A review of the current evidence. *Journal of Clinical Medicine*, *10*(16). https://doi.org/10.3390/jcm10163558

Fuentes-Peñaranda, Y., Labarta-González-Vallarino, A., Arroyo-Bello, E., & Gómez de Quero Córdoba, M. (2025). Global Trends in Diabetic Foot Research (2004–2023): A Bibliometric Study Based on the Scopus Database. *International Journal of Environmental Research and Public Health*, *22*(4), 463. https://doi.org/10.3390/ijerph22040463

Gayathri, K. G., Shinde, P. L., John, S., Sivakumar, K. C., & Mishra, R. (2023). Understanding the Combined Effects of

High Glucose Induced Hyper-Osmotic Stress and Oxygen Tension in the Progression of Tumourigenesis: From Mechanism to Anti-Cancer Therapeutics. *Cells*, *12*(6). https://doi.org/10.3390/cells12060825

González, P., Lozano, P., Ros, G., & Solano, F. (2023). Hyperglycemia and Oxidative Stress: An Integral, Updated and Critical Overview of Their Metabolic Interconnections. *International Journal of Molecular Sciences*, *24*(11). https://doi.org/10.3390/ijms24119352

Hunt, M., Torres, M., Bachar-Wikstrom, E., & Wikstrom, J. D. (2024). Cellular and molecular roles of reactive oxygen species in wound healing. *Communications Biology*, *7*(1), 1–13. https://doi.org/10.1038/s42003-024-07219-w

IDF. (2025). Diabetes Atlas 11th Edition. In *Diabetes Atlas 11th Edition*.

Iosageanu, A., Stefan, L. M., Craciunescu, O., & Cimpean, A. (2024). Anti-Inflammatory and Wound Healing Properties of Different Honey Varieties from Romania and Correlations to Their Composition. *Life*, *14*(9), 1187. https://doi.org/10.3390/life14091187

Ma, Z., Ding, Y., Ding, X., Mou, H., Mo, R., & Tan, Q. (2023). PDK4 rescues high-glucose-induced senescent fibroblasts and promotes diabetic wound healing through enhancing glycolysis and regulating YAP and JNK pathway. *Cell Death Discovery*, *9*(1), 424. https://doi.org/10.1038/s41420-023-01725-2

Mamun, A. Al, Shao, C., Geng, P., Wang, S., & Xiao, J. (2024). Recent advances in molecular mechanisms of skin wound healing and its treatments. *Frontiers in Immunology*, *15*(May), 1395479. https://doi.org/10.3389/fimmu.2024.1395479

Nagy, T., Fisi, V., Frank, D., Kátai, E., Nagy, Z., & Miseta, A. (2019). Hyperglycemia-Induced Aberrant Cell Proliferation; A Metabolic Challenge Mediated by Protein O-GlcNAc Modification. *Cells*, *8*(9), 999. https://doi.org/10.3390/cells8090999

Pasupuleti, V. R., Arigela, C. S., Gan, S. H., Salam, S. K. N., Krishnan, K. T., Rahman, N. A., & Jeffree, M. S. (2020). A Review on Oxidative Stress, Diabetic Complications, and the Roles of Honey Polyphenols. *Oxidative Medicine and Cellular Longevity*, *2020*, 1–16. https://doi.org/10.1155/2020/8878172

Saad, B. (2025). Immunomodulatory and Anti-Inflammatory Properties of Honey and Bee Products. *Immuno*, *5*(2), 19. https://doi.org/10.3390/immuno5020019

Scepankova, H., Combarros-Fuertes, P., Fresno, J. M., Tornadijo, M. E., Dias, M. S., Pinto, C. A., Saraiva, J. A., & Estevinho, L. M. (2021). Role of honey in advanced wound care. In *Molecules*. https://doi.org/10.3390/molecules26164784

Sharma, A., Tate, M., Mathew, G., Vince, J. E., Ritchie, R. H., & De Haan, J. B. (2018). Oxidative stress and NLRP3-inflammasome activity as significant drivers of diabetic cardiovascular complications: Therapeutic implications. *Frontiers in Physiology*, *9*(FEB), 1–15. https://doi.org/10.3389/fphys.2018.00114

Song, J., Zhao, T., Wang, C., Sun, X., Sun, J., & Zhang, Z. (2025). Cell migration in diabetic wound healing: Molecular mechanisms and therapeutic strategies (Review). *International Journal of Molecular Medicine*, *56*(2), 1–26. https://doi.org/10.3892/ijmm.2025.5567

Tashkandi, H. (2021). Honey in wound healing: An updated review. *Open Life Sciences*, *16*(1), 1091–1100. https://doi.org/10.1515/biol-2021-0084

Wilczyńska, A., & Żak, N. (2024). Polyphenols as the Main Compounds Influencing the Antioxidant Effect of Honey—A Review. *International Journal of Molecular Sciences*, *25*(19). https://doi.org/10.3390/ijms251910606

Zhang, S., Ke, Z., Yang, C., Zhou, P., Jiang, H., Chen, L., Li, Y., & Li, Q. (2021). High Glucose Causes Distinct Expression Patterns of Primary Human Skin Cells by RNA Sequencing. *Frontiers in Endocrinology*, *12*(March). https://doi.org/10.3389/fendo.2021.603645

THIS PAGE INTENTIONALLY LEFT BLANK