ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Research Article / Araştırma Makalesi DOI: 10.5281/zenodo.13976728

Evaluation of Wound Healing Potential of Galangin on L929 Mouse Fibroblast Cell Lines Using *In Vitro* Scratch Assay

Galanginin L929 Fare Fibroblast Hücre Hatları Üzerindeki Yara İyileştirme Potansiyelinin *İn Vitro* Çizik Testi Kullanılarak Değerlendirilmesi

Pelin AYDIN[1]
Zeynep KARAKÖY[2]
Hamza HALICI[3]

[1] Department of Anesthesiology and Reanimation, Erzurum City Hospital, Erzurum,Türkiye
[2] Department of Pharmacology, Erzincan Binali Yıldırım University, Faculty of Pharmacy, Erzincan, Türkiye
[3] Department of Hınıs Vocational Training School, Atatürk University, Erzurum, Türkiye

ABSTRACT

Objective: This study aimed to examine the impact of galangin, a compound recognized for its anti-inflammatory and antioxidant properties, on cell proliferation and migration using a scratch wound healing model in the L929 cell line.

Methods: In this study, we investigated the effects of different concentrations of galangin on cell proliferation and viability in the L929 cell line using the MTT method. We then examined the effects of different concentrations of galangin on wound closure in the wound line created by the scratch wound healing test. At 0, 12, 24 and 36 hours, microscopic images of the wound line were taken. Wound closure rates were calculated and a percent wound closure graph was created. At the end of our study, TGF-β levels of all groups were measured using ELISA kits.

Results: According to the viability percentages determined by the MTT method in L929 cells, 10, 25 and 50 µM concentrations of galangin significantly increased cell viability at 24, 48 and 72 hours. In the scratch wound healing model in which these three concentrations of galangin were applied as treatment, it was observed that 25 and 50 µM concentrations of galangin showed a nearly complete closure at the end of the experiment. When the measured TGF-β levels were analyzed, a significant decrease was observed in the galangin-treated groups compared to the control group.

Conclusion: In the *in vitro* scratch wound healing model, the observed reduction in TGF-β levels at end of the experiment in the galangin-treated groups, compared to the control group, suggests that the healing process is nearly completed, resulting in a concomitant decrease in cytokine release. These findings are consistent with the percentage closure rates assessed microscopically across the different treatment groups.

Keywords: Galangin, Migration, Mouse skin fibroblast, Scratch wound assay

ÖZ

Amaç: Bu çalışmada, anti-enflamatuar ve antioksidan özellikleriyle tanınan bir bileşik olan galanginin, L929 hücre hattında çizik yara iyileşme modeli kullanılarak hücre proliferasyonu ve göçü üzerindeki etkisinin incelenmesi amaçlanmıştır.

Yöntemler: Bu çalışmada, farklı galangin konsantrasyonlarının L929 hücre hattında hücre çoğalması ve canlılığı üzerindeki etkilerini MTT yöntemini kullanarak araştırdık. Daha sonra, çizik yara iyileşme testi ile oluşturulan yara hattında farklı galangin konsantrasyonlarının yara kapanması üzerindeki etkilerini inceledik. 0, 12, 24 ve 36. saatlerde yara hattının mikroskobik görüntüleri alındı. Yara kapanma oranları hesaplandı ve yüzde yara kapanma grafiği oluşturuldu. Çalışmamızın sonunda tüm grupların TGF-β seviyeleri ELISA kitleri kullanılarak ölçüldü.

Bulgular: L929 hücrelerinde MTT yöntemi ile belirlenen canlılık yüzdelerine göre, galanginin 10, 25 ve 50 µM konsantrasyonları 24, 48 ve 72. saatlerde hücre canlılığını önemli ölçüde artırmıştır. Galanginin bu üç konsantrasyonunun tedavi olarak uygulandığı çizik yara iyileşme modelinde, galanginin 25 ve 50 µM konsantrasyonlarının deney sonunda neredeyse tamamen kapanma gösterdiği gözlemlenmiştir. Ölçülen TGF-β seviyeleri analiz edildiğinde, galangin uygulanan gruplarda kontrol grubuna kıyasla anlamlı bir düşüş gözlenmiştir.

Sonuç: *İn vitro* çizik yara iyileşme modelinde, galangin ile tedavi edilen gruplarda deney sonunda TGF-β seviyelerinde kontrol grubuna kıyasla gözlenen azalma, iyileşme sürecinin neredeyse tamamlandığını ve sitokin salınımında eşzamanlı bir azalmaya neden olduğunu göstermektedir. Bu bulgular, farklı tedavi gruplarında mikroskobik olarak değerlendirilen yüzde kapanma oranlarıyla tutarlıdır.

Anahtar Kelimeler: Galangin, migrasyon, fare deri fibroblastı, çizik yara deneyi

Geliş Tarihi/Received 20.09.2024
Kabul Tarihi/Accepted 19.10.2024
Yayın Tarihi/Publication Date 28.10.2024

Sorumlu Yazar/Corresponding author: Hamza Halıcı
E-mail: hamzahalici@atauni.edu.tr

Cite this article: Aydın, P., Karaköy, Z., & Halıcı, H. (2024). Evaluation of Wound Healing Potential of Galangin on L929 Mouse Fibroblast Cell Lines Using In Vitro Scratch Assay. *Current Research in Health Sciences*, 1(3): 99-104.

Introduction

The skin is responsible for regulating homeostasis and protecting the body against physical stimuli from the environment, pathogens and dehydration (Proksch et al., 2008). The skin can be injured for various reasons. After injury, wound healing mechanisms are activated to repair and reconstruct the damaged tissue (De Jesus et al., 2016). The wound healing process can be delineated into overlapping stages, comprising inflammation, proliferation, and remodeling (Yan et al., 2024).

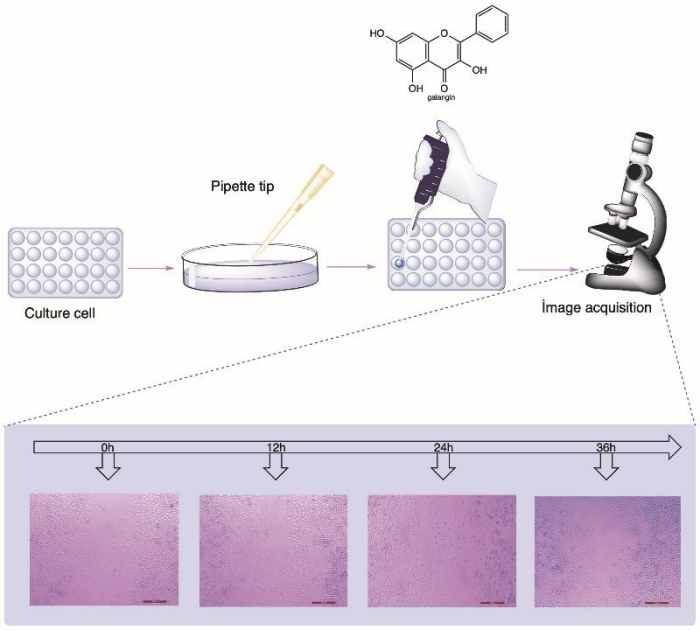

Figure 1. The process of making wound

The inflammation process starts with homeostasis, which prevents blood loss from the wound. Neutrophils traveling to the site of injury provide a favorable environment for wound healing and remove bacteria and debris (Berman et al., 2017). During the proliferative phase, keratinocytes, fibroblasts, and endothelial cells undergo migration and proliferation. This process is initiated by the release of growth factors, including transforming growth factor-β (TGF-β) and vascular endothelial growth factor (VEGF) (Bielefeld et al., 2013). The remodeling phase represents the concluding step of wound healing, necessitating a careful balance between the apoptosis of existing cells and the proliferation of new cells (Wang et al., 2018). Despite its complexity, recovery is often successful. If the cascade goes awry at any point in the healing process, pathological conditions such as non-healing wounds or fibrosis can occur (Bochaton-Piallat et al., 2016; Darby & Hewitson, 2007). It is critical that type III collagen, initially secreted by myofibroblasts stimulated by TGF-β, is degraded and replaced by type-1 collagen. Any deviation during this stage can result in either excessive wound healing or the formation of chronic wounds (Plikus et al., 2017; Tsai et al., 2018).

A review of recent studies reveals that current treatments aimed at promoting wound healing and preventing chronic wound development remain inadequate. Consequently, there is a need for novel pharmacological agents in the treatment of wounds (El Ayadi et al., 2020). Galangin is a natural flavonoid primarily extracted from propolis and the roots of Alpinia officinarum Hance (Rampogu et al., 2021). It is also used in modern clinical therapy due to its antioxidant, anti-inflammatory and proapoptosis effects (Ru et al., 2021; Yu et al., 2018). In addition, bioflavonoids have been reported to alleviate fibrosis in kidney and kidney organs (Cao et al., 2018). A recent study in rabbits suggested that galangin may potentially prevent hypertrophic scar formation (Ru et al., 2021). Numerous studies have been conducted to investigate the therapeutic effect of galangin, but little is known about its effect on wound healing. In addition, no studies are showing the effect of galangin on wound healing at the cellular level. Considering the bioactivity and pharmacological effects of galangin, it is suggested that it may have positive effects on the wound healing process and encourages research.

In summary, this study aimed to explore the effects of galangin on cell viability in L929 fibroblast cells, as well as to evaluate its role in fibroblast migration and wound closure during

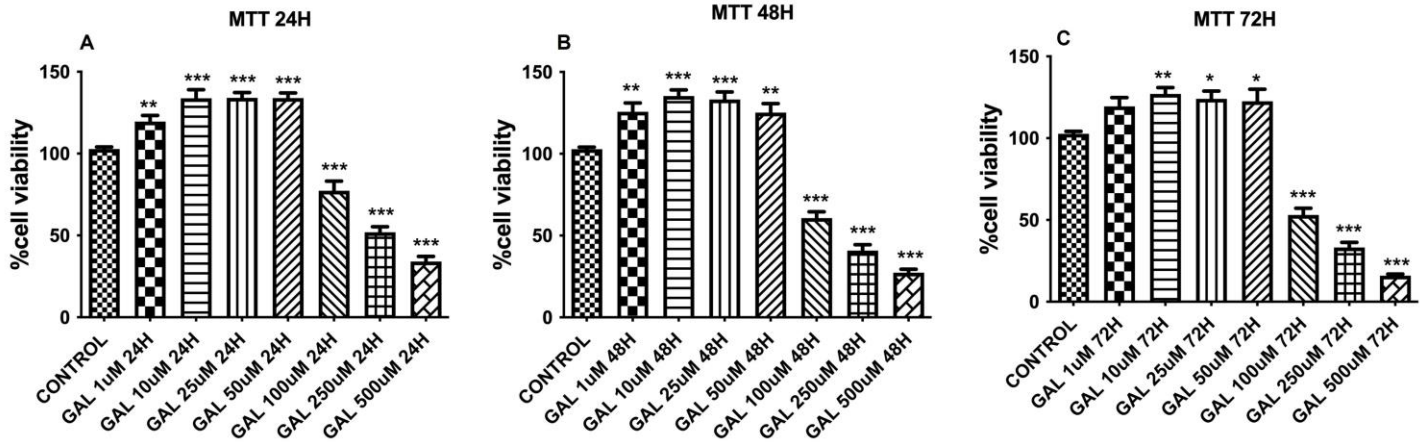

Figure 2. Cell viability results obtained from MTT test. * $p<.05$, ** $p<.01$ and *** $p<.001$ according to Tukey's post-hoc test.

the wound healing process.

Methods

Cell Culture

The L929 cell line, acquired from the American Type Culture Collection (ATCC, USA) and cryopreserved in a Cryotube, was thawed from liquid nitrogen storage and transferred into a T75 cm^2 flask containing DMEM supplemented with 10% fetal bovine serum (FBS). The flask was subsequently incubated at 37°C in a humidified atmosphere with 5% CO2.

Assessment of Cell Viability

A total of 5 × 10^3 cells were added to each well of a 96-well plate. The cells were incubated for 24 hours to allow adhesion to the bottom of the wells. At the end of this period, galangin was dissolved in DMSO, and different concentrations (1, 10, 25, 50, 100, 250, and 500 µM) were prepared. At 24, 48, and 72 hours, measurements were taken using the MTT method with a microplate reader (Epoch Microplate Spectrophotometer, BioTek, USA) at an absorbance wavelength of 570 nm. Cell viability rates were analyzed by comparing them to control wells.

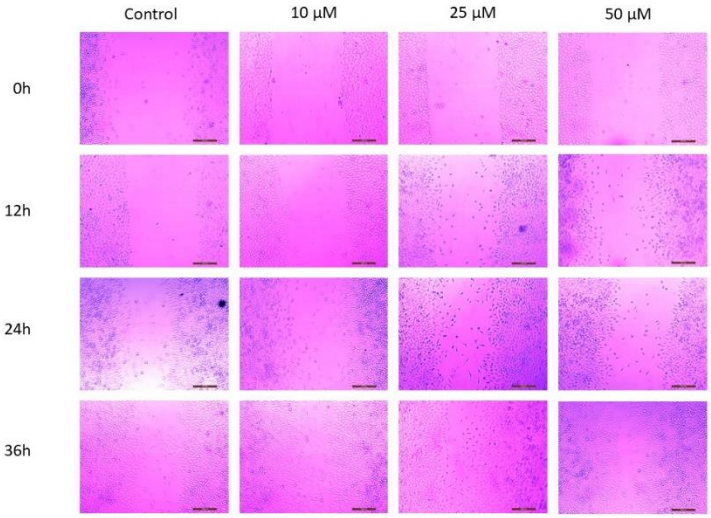

Figure 3. Photographs of scratch test after 0, 12, 24 and 36 hours.

Scratch Wound Healing Experiment

A total of 2 × 105 cells were added to the wells of a six-well culture plate and incubated overnight. An *in vitro* wound model was established by creating a scratch across the monolayer cell layer formed by fibroblast cells in each well of a six-well culture plate using a sterile pipette tip in a single motion. During the scratching procedure, the upper medium was removed to eliminate debris, and the wells were washed with sterile phosphate-buffered saline (PBS) before adding fresh medium. A healthy group without a wound line and a control group with a wound line were formed. Galangin was applied to cells at 10, 25 and 50 µM concentrations. Images of all wells were taken using a Leica Inverted Microscope (Leica, DMIL LED) before and at 12, 24 and 36 hours after galangin treatment. Wound closure rates were calculated using the following formula (Figure 1).

% wound closure=[(At=oh-At=Δh)/At=0h]*100

At=0h: Wound length measured at 0th hour (µm)

At=Δh: Wound length measured at Δth hour (µm)

Determination of TGF-β1 Amount

At the end of the experiment, the supernatants of all experimental groups in the 6-well plate were taken. TGF-β1 levels were measured using ELISA kits on Epoch Spectrophotometer System and Take3 Plate. The ELISA procedure was conducted following the steps outlined in the kit protocol.

Statistical Analysis

The data were analyzed using IBM SPSS version 27 (27 (IBM SPSS Corp., Armonk, NY, USA). Statistical analysis was performed using One-way ANOVA, followed by Tukey's post-hoc multiple comparison test. Results are reported as the arithmetic mean ± standard deviation, with a p-value of <0.05 considered statistically significant.

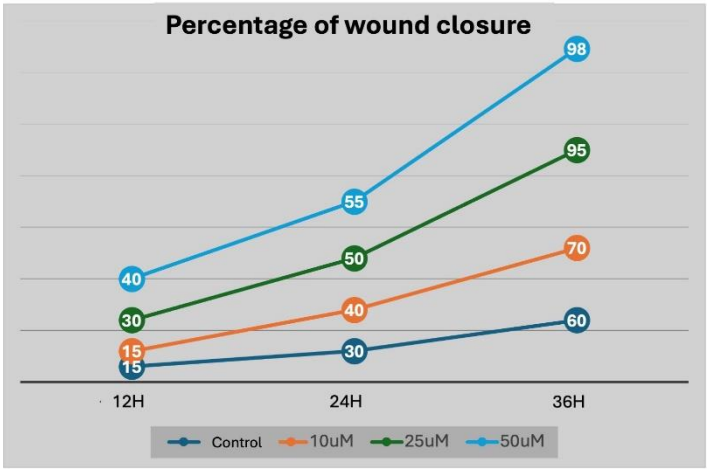

Figure 4. Wound Closure Percentage at 12, 24, and 36 Hours

Results

When the MTT findings of our study are examined; it is seen that galangin concentrations of 1 µM (p<.01), 10 µM (p<.001), 25 µM (p<.001) and 50 µM (p<.001) significantly increased the percent viability of the control group at 24 hours. Galangin concentrations of 100, 250 and 500 µM significantly decreased the percent viability compared to the control group (p<.001) (Figure 2A). At 48 h; 1 µM (p<.01), 10 µM (p<.001), 25 µM (p<.001) and 50 µM (p<.01) galangin concentrations significantly increased the percent viability compared to the control group. When compared to the control group, 100, 250 and 500 µM concentrations of galangin significantly decreased the percent viability (p<.001), similar to the results obtained at 24 h (Figure 2B). At 72nd; 10 (p<.01), 25 (p<.05) and 50 µM (p<.05) concentrations of galangin significantly increased the percent

viability compared to the control group. Compared to the control group, 100, 250 and 500 µM concentrations of galangin significantly decreased percent viability (p<.001), similar to the findings at 24 and 48 hours (Figure 2C).

In the scratch wound healing model, we created in our study, images of the wells were taken at 0, 12, 24 and 36 hours and wound closure percentages were calculated according to the formula mentioned above (Figure 3,4). When the graph is analyzed, the wound closure percentages calculated from the images taken at the 12th hour are higher in the groups treated with 10, 25 and 50 µM galangin compared to the control group. The 50 µM galangin treatment group showed a higher percentage of closure compared to the other groups. When the wound closure percentages calculated from the images taken at 24 hours are examined, it is seen that the closure percentage is 50% in the 25 µM galangin treated group and 55% in the 50 µM galangin treated group. At the end of the 36th hour, a nearly complete closure percentage was observed in the 25 and 50 µM galangin-treated groups.

When the TGF-β levels measured from the supernatants taken at the end of the experiment were analyzed; it was observed that TGF-β levels were significantly increased in the control group compared to the healthy group (p<.05). TGF-β levels were substantially lower in the groups treated with 25 and 50 µM galangin compared to the control group (p<.001) (Figure 5).

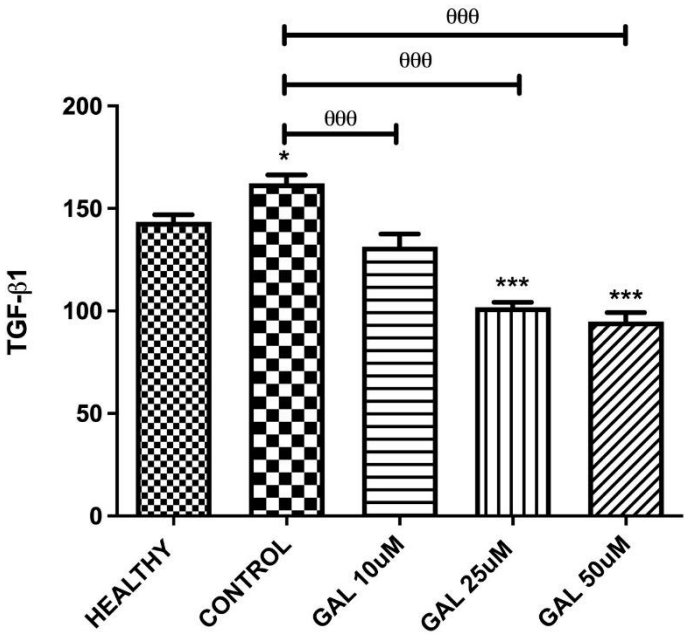

Figure 5. TGF-β1 ELISA results at 36th hour of scratch test. *, θ p<.05, **, θθ p<.01 and ***, θθθ p<.001, according to Tukey's post-hoc test.

Discussion

In this study, we investigated the effect of galangin on cell viability in L929 skin fibroblast cells and examined its effect on cell migration in scratch wound healing assay. When the findings of our study are examined, it is seen that in the dose study with galangin; 10, 25, and 50 µM concentrations significantly increased the viability of L929 skin fibroblast cells. In the scratch wound healing test performed with these doses, it was shown that at the end of 36 hours, 25 and 50 µM concentrations of galangin provided nearly complete closure. TGF-β levels measured at the end of the experiment showed a dose-dependent decrease in the treatment groups compared to the control.

Fibroblast cells are among the most extensively researched cell types in the context of wound healing (Borges et al., 2017; Teplicki et al., 2018). The MTT assay is commonly employed to assess cell viability, making it a preferred method for analyzing both cell proliferation and cytotoxic effects (Cangul et al., 2020; Ozdemir et al., 2009). In our study, we evaluated the impact of galangin at various concentrations (1, 10, 25, 50, 100, 250, and 500 µM) on the viability of L929 skin fibroblast cells using the MTT assay. Measurements taken at 24, 48, and 72 hours indicated that the 10, 25, and 50 µM concentrations of galangin significantly increased cell viability compared to the control group.

The scratch wound assay is a widely used, reproducible method for assessing key parameters of cell migration, including velocity, persistence, and polarity. Once the cells have reached confluence on the bottom of the plate, a thin wound is created using a sterile pipette tip (Cory, 2011). Numerous studies have employed this method to investigate wound healing (Jagiello et al., 2023; Pinto et al., 2019). Wound healing occurs in all tissues and organs of the body, with many of these repair processes being common across various tissue types. While the healing process is continuous, the physiological events occurring within the wound and the surrounding tissue can be categorized into distinct stages (Richardson, 2004). The wound healing process can be delineated into overlapping stages, comprising inflammation, proliferation, and remodeling (Yan et al., 2024). If any disruption occurs within the cascade during the healing process, it may lead to pathological conditions such as chronic non-healing wounds or fibrosis (Bochaton-Piallat et al., 2016; Darby & Hewitson, 2007). When the studies on wound healing are examined; positive results have been obtained in studies with substances with antioxidant and anti-inflammatory properties (Hecker et al., 2022; Kant et al., 2015). In our study, the effects of galangin, which is known to have antioxidant and anti-inflammatory properties, on wound healing were investigated. In an *in vivo* study, Ru et al. reported that galangin may potentially prevent hypertrophic scar formation (Ru et al., 2021). In our study, in the scratch wound healing model, 25 and 50 µM concentrations of galangin provided a nearly complete closure of the wound line at the end of the experiment. It was shown *in vitro* that galangin has positive effects on wound healing.

It is well known that growth factors and cytokines have important roles in all stages of wound healing (Everts et al., 2006). TGF-β1 plays a pivotal role in wound healing by facilitating key processes such as inflammation, angiogenesis, re-

epithelialization, and connective tissue regeneration. Research has indicated that its expression significantly rises at the initial stages of injury (Kane et al., 1991; Kopecki et al., 2007). TGFβ plays a pivotal role in maintaining skin homeostasis by inhibiting keratinocyte proliferation and regulating their differentiation. Upon wounding and disruption of the epidermal barrier, TGFβ signaling continues to exert significant effects on keratinocyte function and the regulation of wound re-epithelialization (Ramirez et al., 2014). *İn vitro* studies demonstrate that TGF-β1 plays a crucial role in the initiation of granulation tissue formation by upregulating the expression of genes related to extracellular matrix formation, such as fibronectin, fibronectin receptors, collagen, and protease inhibitors (Greenwel et al., 1997; Mauviel et al., 1996; White et al., 2000). Furthermore, *in vitro* studies indicate that TGF-β1 contributes to wound contraction by promoting fibroblast-mediated contraction of the collagen matrix (Meckmongkol et al., 2007). In the remodeling phase, which is the last phase of wound healing, collagen Type-3 is converted to Type-1 and TGF- β differentiates myofibroblasts and closes the wound (Desmouliere et al., 1993; Hosokawa et al., 2003; Ronnov-Jessen & Petersen, 1993). At the end of the wound-healing process, growth factors and cytokines decrease. The reduction in these growth factors and cytokines suggests that the wound-healing process has been completed (Nogueira et al., 2020). In our study, the TGF-β1 levels measured after the experiment were significantly reduced in the groups treated with 25 and 50 µM galangin compared to the control group. Additionally, in line with the microscopic observations, wound closure was nearly complete in these groups, suggesting that galangin effectively supported the healing process.

Conclusion

In the *in vitro* scratch wound healing model, the observed decrease in TGF-β levels at the end of the experiment in the galangin-treated groups, relative to the control group, indicates that the healing process is approaching completion, which is associated with a reduction in cytokine release. These results are in line with the microscopically measured percent closure rates by group. However, this effect of galangin should also be evaluated by *in vivo* experiments. Experimental and clinical studies are needed to evaluate the effects and mechanisms of galangin on wound healing.

Etik Komite Onayı: Hücre Kültürü çalışması olduğundan etik komite onamına ihtiyaç yoktur.
Hakem Değerlendirmesi: Dış bağımsız.
Yazar Katkıları: Fikir – P.A.; Tasarım – P.A.; Denetleme – P.A.; Kaynaklar – Z.K.; Veri Toplanması ve/veya İşlemesi – Z.K.; Analiz ve/ veya Yorum- Z.K., H.H.; Literatür Taraması – P.A., H.H.; Yazıyı Yazan- P.A., H.H.; Eleştirel İnceleme – P.A., H.H.
Çıkar Çatışması: Yazarlar, çıkar çatışması olmadığını beyan etmiştir.
Finansal Destek: Yazarlar, bu çalışma için finansal destek almadığını beyan etmiştir.

Ethics Committee Approval: Since it is a cell culture study, ethics committee approval is not required.
Peer-review: Externally peer-reviewed.
Author Contributions: Concept - P.A.; Design - P.A.; Supervision - P.A.; Resources - Z.K.; Materials - Z.K.; Data Collection and/or Processing - Z.K.; Analysis and/or Interpretation – Z.K., H.H.; Literature Search – P.A., H.H.; Writing Manuscript – P.A., H.H.; Critical Review – P.A., H.H..
Conflict of Interest: The authors have no conflicts of interest to declare.
Financial Disclosure: The authors declared that this study has received no financial support.

References

Berman, B., Maderal, A., & Raphael, B. (2017). Keloids and Hypertrophic Scars: Pathophysiology, Classification, and Treatment. *Dermatologic Surgery*, *43*, S3-S18. https://doi.org/10.1097/Dss.0000000000000819

Bielefeld, K. A., Amini-Nik, S., & Alman, B. A. (2013). Cutaneous wound healing: recruiting developmental pathways for regeneration. *Cellular and Molecular Life Sciences*, *70*(12), 2059-2081. https://doi.org/10.1007/s00018-012-1152-9

Bochaton-Piallat, M. L., Gabbiani, G., & Hinz, B. (2016). The myofibroblast in wound healing and fibrosis: answered and unanswered questions. *F1000Res*, *5*. https://doi.org/10.12688/f1000research.8190.1

Borges, G. A., Elias, S. T., da Silva, S. M., Magalhaes, P. O., Macedo, S. B., Ribeiro, A. P., & Guerra, E. N. (2017). In vitro evaluation of wound healing and antimicrobial potential of ozone therapy. *J Craniomaxillofac Surg*, *45*(3), 364-370. https://doi.org/10.1016/j.jcms.2017.01.005

Cangul, S., Adiguzel, O., & Tekin, S. (2020). Comparison of Cytotoxicity of Four Different Adhesive Materials Before and After Polymerisation. *Oral Health & Preventive Dentistry*, *18*(1), 43-51. https://doi.org/10.3290/j.ohpd.a43940

Cao, Y. C., Hu, J. L., Sui, J. Y., Jiang, L. M., Cong, Y. K., & Ren, G. Q. (2018). Quercetin is able to alleviate TGF--induced fibrosis in renal tubular epithelial cells by suppressing miR-21. *Experimental and Therapeutic Medicine*, *16*(3), 2442-2448. https://doi.org/10.3892/etm.2018.6489

Cory, G. (2011). Scratch-Wound Assay. *Cell Migration: Developmental Methods and Protocols, Second Edition*, *769*, 25-30. https://doi.org/10.1007/978-1-61779-207-6_2

Darby, I. A., & Hewitson, T. D. (2007). Fibroblast differentiation in wound healing and fibrosis. *International Review of Cytology - a Survey of Cell Biology, Vol 257*, *257*, 143-+. https://doi.org/10.1016/S0074-7696(07)57004-X

De Jesus, A. M., Aghvami, M., & Sander, E. A. (2016). A Combined In Vitro Imaging and Multi-Scale Modeling System for Studying the Role of Cell Matrix Interactions in Cutaneous Wound Healing. *Plos One*, *11*(2). https://doi.org/ARTN e014825410.1371/journal.pone.0148254

Desmouliere, A., Geinoz, A., Gabbiani, F., & Gabbiani, G. (1993). Transforming growth factor-beta 1 induces alpha-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. *J Cell Biol*, *122*(1), 103-111. https://doi.org/10.1083/jcb.122.1.103

El Ayadi, A., Jay, J. W., & Prasai, A. (2020). Current Approaches Targeting the Wound Healing Phases to Attenuate Fibrosis and Scarring. *Int J Mol Sci*, *21*(3). https://doi.org/10.3390/ijms21031105

Everts, P. A., Knape, J. T., Weibrich, G., Schonberger, J. P., Hoffmann, J., Overdevest, E. P., Box, H. A., & van Zundert, A. (2006). Platelet-rich plasma and platelet gel: a review. *J Extra Corpor Technol*, *38*(2), 174-187. https://www.ncbi.nlm.nih.gov/pubmed/16921694

Greenwel, P., Inagaki, Y., Hu, W., Walsh, M., & Ramirez, F. (1997). Sp1 is required for the early response of alpha 2(I) collagen to

transforming growth factor-beta 1. *Journal of Biological Chemistry*, *272*(32), 19738-19745. https://doi.org/DOI10.1074/jbc.272.32.19738

Hecker, A., Schellnegger, M., Hofmann, E., Luze, H., Nischwitz, S. P., Kamolz, L. P., & Kotzbeck, P. (2022). The impact of resveratrol on skin wound healing, scarring, and aging. International Wound Journal, 19(1), 9-28. https://doi.org/10.1111/iwj.13601

Hosokawa, R., Nonaka, K., Morifuji, M., Shum, L., & Ohishi, M. (2003). TGF-beta 3 decreases type I collagen and scarring after labioplasty. *J Dent Res*, *82*(7), 558-564. https://doi.org/10.1177/154405910308200714

Jagiello, K., Uchanska, O., Matyja, K., Jackowski, M., Wiatrak, B., Kubasiewicz-Ross, P., & Karuga-Kuzniewska, E. (2023). Supporting the Wound Healing Process-Curcumin, Resveratrol and Baicalin in In Vitro Wound Healing Studies. *Pharmaceuticals*, *16*(1). https://doi.org/ARTN 8210.3390/ph16010082

Kane, C. J. M., Hebda, P. A., Mansbridge, J. N., & Hanawalt, P. C. (1991). Direct Evidence for Spatial and Temporal Regulation of Transforming Growth-Factor Beta-1 Expression during Cutaneous Wound-Healing. *Journal of Cellular Physiology*, *148*(1), 157-173. https://doi.org/DOI 10.1002/jcp.1041480119

Kant, V., Gopal, A., Kumar, D., Pathak, N. N., Ram, M., Jangir, B. L., Tandan, S. K., & Kumar, D. (2015). Curcumin-induced angiogenesis hastens wound healing in diabetic rats. *Journal of Surgical Research*, *193*(2), 978-988. https://doi.org/10.1016/j.jss.2014.10.019

Kopecki, Z., Luchetti, M. M., Adams, D. H., Strudwick, X., Mantamadiotis, T., Stoppacciaro, A., Gabrielli, A., Ramsay, R. G., & Cowin, A. J. (2007). Collagen loss and impaired wound healing is associated with c-Myb deficiency. *Journal of Pathology*, *211*(3), 351-361. https://doi.org/10.1002/path.2113

Mauviel, A., Chung, K. Y., Agarwal, A., Tamai, K., & Uitto, J. (1996). Cell-specific induction of distinct oncogenes of the Jun family is responsible for differential regulation of collagenase gene expression by transforming growth factor-beta in fibroblasts and keratinocytes. *Journal of Biological Chemistry*, *271*(18), 10917-10923. https://doi.org/DOI 10.1074/jbc.271.18.10917

Meckmongkol, T. T., Harmon, R., McKeown-Longo, P., & Van De Water, L. (2007). The fibronectin synergy site modulates TGF-beta-dependent fibroblast contraction. *Biochem Biophys Res Commun*, *360*(4), 709-714. https://doi.org/10.1016/j.bbrc.2007.06.121

Nogueira, B. C. F., Campos, A. K., Alves, R. S., Sarandy, M. M., Novaes, R. M. D., Esposito, D., & Goncalves, R. V. (2020). What Is the Impact of Depletion of Immunoregulatory Genes on Wound Healing? A Systematic Review of Preclinical Evidence. *Oxidative Medicine and Cellular Longevity*, *2020*. https://doi.org/Artn886295310.1155/2020/8862953

Ozdemir, K. G., Yilmaz, H., & Yilmaz, S. (2009). In vitro evaluation of cytotoxicity of soft lining materials on L929 cells by MTT assay. *J Biomed Mater Res B Appl Biomater*, *90*(1), 82-86. https://doi.org/10.1002/jbm.b.31256

Pinto, B. I., Cruz, N. D., Lujan, O. R., Propper, C. R., & Kellar, R. S. (2019). Scratch Assay to Demonstrate Effects of Arsenic on Skin Cell Migration. *Jove-Journal of Visualized Experiments*(144). https://doi.org/ARTNe5883810.3791/58838

Plikus, M. V., Guerrero-Juarez, C. F., Ito, M., Li, Y. R., Dedhia, P. H., Zheng, Y., Shao, M., Gay, D. L., Ramos, R., Hsi, T. C., Oh, J. W., Wang, X. J., Ramirez, A., Konopelski, S. E., Elzein, A., Wang, A., Supapannachart, R. J., Lee, H. L., Lim, C. H., . . . Cotsarelis, G. (2017). Regeneration of fat cells from myofibroblasts during wound healing. *Science*, *355*(6326), 748-+. https://doi.org/10.1126/science.aai8792

Proksch, E., Brandner, J. M., & Jensen, J. M. (2008). The skin: an indispensable barrier. *Experimental Dermatology*, *17*(12), 1063-1072. https://doi.org/10.1111/j.1600-0625.2008.00786.x

Ramirez, H., Patel, S. B., & Pastar, I. (2014). The Role of TGFβ Signaling in Wound Epithelialization. *Advances in Wound Care*, *3*(7), 482-491. https://doi.org/10.1089/wound.2013.0466

Rampogu, S., Gajula, R. G., & Lee, K. W. (2021). A comprehensive review on chemotherapeutic potential of galangin. *Biomedicine & Pharmacotherapy*, *141*. https://doi.org/ARTN 11180810.1016/j.biopha.2021.111808

Richardson, M. (2004). Acute wounds: an overview of the physiological healing process. *Nurs Times*, *100*(4), 50-53. https://www.ncbi.nlm.nih.gov/pubmed/14974265

Ronnov-Jessen, L., & Petersen, O. W. (1993). Induction of alpha-smooth muscle actin by transforming growth factor-beta 1 in quiescent human breast gland fibroblasts. Implications for myofibroblast generation in breast neoplasia. *Lab Invest*, *68*(6), 696-707. https://www.ncbi.nlm.nih.gov/pubmed/8515656

Ru, Z., Hu, Y., Huang, S. H., Bai, L., Zhang, K., & Li, Y. (2021). Bioflavonoid Suppresses Hypertrophic Scar Formation by the TGF-/Smad Signaling Pathway. *Evidence-Based Complementary and Alternative Medicine*, *2021*. https://doi.org/Artn 244483910.1155/2021/2444839

Teplicki, E., Ma, Q., Castillo, D. E., Zarei, M., Hustad, A. P., Chen, J., & Li, J. (2018). The Effects of Aloe vera on Wound Healing in Cell Proliferation, Migration, and Viability. *Wounds*, *30*(9), 263-268. https://www.ncbi.nlm.nih.gov/pubmed/30256753

Tsai, H.-W., Wang, P.-H., & Tsui, K.-H. (2018). Mesenchymal stem cell in wound healing and regeneration. In (Vol. 81, pp. 223-224): LWW.

Wang, P.-H., Huang, B.-S., Horng, H.-C., Yeh, C.-C., & Chen, Y.-J. (2018). Wound healing. *Journal of the Chinese Medical Association*, *81*(2), 94-101. https://doi.org/10.1016/j.jcma.2017.11.002

White, L. A., Mitchell, T. I., & Brinckerhoff, C. E. (2000). Transforming growth factor β inhibitory element in the rabbit matrix metalloproteinase-1 (collagenase-1) gene functions as a repressor of constitutive transcription. *Biochimica Et Biophysica Acta-Gene Structure and Expression*, *1490*(3), 259-268. https://doi.org/Doi 10.1016/S0167-4781(00)00002-6

Yan, Y., You, A. J., Chen, X. X., Huang, W. Y., Lu, X. T., Gu, C. J., Ye, S., Zhong, J., Huang, H. T., Zhao, Y., Li, Y., & Li, C. (2024). (+)4-cholesten-3-one/sodium alginate/gelatin hydrogel for full-thickness wound repair and skin regeneration. *Biomedical Materials*, *19*(5). https://doi.org/ARTN 05502610.1088/1748-605X/ad6966

Yu, S., Gong, L. S., Li, N. F., Pan, Y. F., & Zhang, L. (2018). Galangin (GG) combined with cisplatin (DDP) to suppress human lung cancer by inhibition of STAT3-regulated NF-κB and Bcl-2/Bax signaling pathways. *Biomedicine & Pharmacotherapy*, *97*, 213-224. https://doi.org/10.1016/j.biopha.2017.10.059