Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2022 Feb 07; 10(A):256-261.
https://doi.org/10.3889/oamjms.2022.8177
eISSN: 1857-9655
Category: *A - Basic Sciences*
Section: *Pharmacology*

Low-dose Indonesian *Aloe vera* Increases Viability and Migration of the Fibroblast: An *In Vitro* Study

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Edited by: Sinisa Stojanoski
Citation: Rizqi J, Fitriawan AS. Low-dose Indonesian *Aloe vera* Increases Viability and Migration of the Fibroblast: An *In vitro* Study. Open-Access Maced J Med Sci. 2022 Feb 07; 10(A):256-261. https://doi.org/10.3889/oamjms.2022.8177
Keywords: *Aloe vera*; Viability, Migration, Wound scratch assay; Wound healing
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Received: 07-Dec-2021
Revised: 24-Jan-2022
Accepted: 28-Jan-2022

Funding: This study was fully funded by the Ministry of Research and Technology/National Research and Innovation Agency (Menristek-BRIN) of the Republic of Indonesia
Competing Interest: The authors have declared that no competing interest exists

Abstract

BACKGROUND: Important stages in wound healing involve homeostasis, inflammation, proliferation, and remodeling phases. Fibroblasts are essential factors in the healing pathway through the process of cell proliferation and migration. Aloe vera contains various active compounds used for anti-inflammatory, antimicrobial, immunomodulatory, anticancer, and wound healing.

AIM: This study aimed to evaluate the effect of A. vera on the viability and migration of fibroblast cells.

MATERIALS AND METHODS: Fibroblasts were cultured in a monolayer with Dulbecco's Modified Eagle Medium containing 10% fetal bovine serum, 1% pinstripe, and 0.5% fungizone. We use fresh *A. vera* leaves extracted with 95% ethanol. Cell viability will be evaluated using the MTT test and microscopic evaluation. Cell migration was tested using an *in vitro* wound scratch assay and analyzed with ImageJ software.

RESULTS: *A. vera* stimulated cell viability compared to control ($p < 0.05$). Administration of *A. vera* does not change shape and is not toxic to fibroblasts. *A. vera* stimulated cell migration at doses of 250, 125, 50, and 5 μg/mL compared to control after 24 h of intervention. At 48 h incubation, migration doses of 250, 50, and 5 μg/mL were higher than control ($p < 0.05$).

CONCLUSIONS: *A. vera* extract may effectively wound healing by increasing viability and migration of fibroblast cells.

Introduction

The skin is a protection for the human body from infection and extrinsic trauma. Wounds can be described as a breakdown of the anatomic and functional integrity of the skin epithelial tissue [1]. The wound healing process is very complex, including physiological, cellular, and molecular events involving the homeostatic, inflammatory, proliferative, and remodeling to repair tissue damage [2]. The homeostatic phase begins after injury, platelets, and the blood coagulation cascade causes fibrin clotting to advance to the next step [3]. Neutrophils start to migrate to the wound bed, starting an inflammatory phase that acts as phagocytosis [4]. Macrophages contribute to the release of platelet-derived growth factor and tumor growth factor (TGF-β), triggering the migration of fibroblast cells to the wound bed to initiate the proliferative phase [5].

The proliferative phase plays an essential role in the formation of granulation tissue and angiogenesis. Fibroblasts are the primary cells in the proliferative phase, producing extracellular cell matrix (ECM) such as collagen, proteoglycans, glycoproteins to replace fibrin clots, and accelerate wound healing [6]. Fibroblasts also produce matrix proteins such as vascular endothelial growth factor (VEGF), which play an essential role in angiogenesis [7]. The remodeling phase maintains a balance between ECM degradation and synthesis, regulated by matrix metalloproteinase (MMP) until complete healing is achieved [8].

Wound management aims to heal wounds with minimal pain, scarring, and shorten healing time. The latest treatment principle is to provide a moist environment for the damage using the proper dressing [9]. A moist wound environment will accelerate the proliferation and migration of fibroblast cells [10], [11]. In general, many dressings that have been used, such as silver and alginate, function to create a moist environment in the wound area and have anti-inflammatory and antimicrobial effects [12]. However, this type of dressing requires a very high cost, and it is difficult for people in the area to obtain it [13]. Therefore, we need alternative natural ingredients that are readily available and more economical, such as *Aloe vera*.

A. vera is a valuable medicinal plant for wound healing and has biological activities such as antifungal, anti-inflammatory, immunomodulatory, and antibacterial effects [14], [15]. *A. vera* is non-toxic and does not affect the morphology of fibroblast cells [16], [17], has bioactive compounds such as acetylated mannans, polymannans, anthraquinone C-glycosides, anthrones,

anthraquinones, and lectins which are therapeutically helpful [15]. Aloin in *A. vera* can induce increased expression of TGF-β1, VEGF [18], and increase proliferation, migration of endothelial cells, and fibroblasts to accelerate wound healing [19].

Fibroblasts are the most abundant cells in connective tissue that plays an essential role in wound healing [20]. Mouse fibroblast cells have been used to model eukaryotic cells similar to human fibroblasts [21]. The purpose of this study was to explore the effect of local Indonesian *A. vera* on cell viability and migration, which was evaluated through the *microculture tetrazolium test* (MTT) test and wound scratch assay.

Materials and Methods

Collection of plant materials and plant extracts

A. vera was obtained from the Self-help Agricultural and Rural Training Center (P4S) in Yogyakarta, Indonesia. Extracts were made using a method that has been modified by previous researchers [18]. *A. vera* leaves were washed and then dried in an oven at 60°C. The dried leaves are then blended into a fine powder. *A. vera* extract preparation was macerated with 95% ethanol at room temperature for 24 h, then filtered with Whatman paper no. 1. The extract was concentrated using a rotary evaporator, then freeze-dried at −55°C, then transferred to a bottle and stored at −8°C.

To obtain a stock solution

The ethanol extract of *A. vera* was dissolved with 1% dimethyl sulfoxide (DMSO; SIGMA®; St. Louis, MO, USA). The stock solution was then diluted into a growth medium (Dulbecco's Modified Eagle Medium [DMEM]) to test the concentrations, namely, 500, 250, 125, and 50 and 5 μg/mL. ALGISITE-M (Smith & Nephew®) was used as the control group. He made the Algisite-M® test by cutting the dressing in 1 cm^2 size and then incubating it in 4 mL of growth media in an incubator at 37°C for 24 h.

Cell culture

The NIH 3T3 cell line used in this study was obtained from the Pharmacology and Therapeutics Laboratory, Universitas Gadjah Mada, Indonesia. This research was conducted after obtaining approval from the research ethics committee of the Integrated Research and Testing Laboratory (LPPT), Universitas Gadjah Mada (code of ethical conduct: 00012/04/LPPT/III/2020). Fibroblast cells were cultured and well cared for. Cells were cultured under standard conditions, with DMEM containing 10% fetal bovine serum (FBS, Sigma), 1% Pen-Strep, and 0.5% fungizone (GIBCO®, Grand Island, NY, USA) in an incubator in 5% CO^2 at 37°C. The culture medium was changed 3 times a week. Trypsin 1% was used to separate fibroblast cells from culture wells.

Fibroblast 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assays

NIH 3T3 cells, $1x10^4$ were inserted into a 96-well microplate (100 L/well) for 24 h. The media was replaced with the tested concentrations of 500, 250, 125, 50, and 5 μg/mL, Algisite-M® as a control media, incubated again for 24 and 48 h. Each media well was replaced with 10 L of MTT reagent and incubated at 37 °C for 4–6 h. The MTT was then discarded, and the formazan crystal file dissolved in 100 L sodium dodecyl sulfate (SDS) was incubated at room temperature for 12 h. Optical cell density was measured using an enzyme-linked immunosorbent assay (ELISA) at a wavelength of 595 nm. The effect of *A. vera* extract on cell viability was calculated by calculating the percentage of cell viability = (Absorbance Treatment–Absorbance Media)/(Absorbance control cells–Absorbance Media) x 100%.

In vitro wound scratch assay

The scratch assay was used to determine the migration rate of fibroblast cells. The fibroblast migration test was carried out by modifying the technique used by Liang [22]. 5×10^4 NIH3T3 cells were placed in a 24-well microplate and incubated in complete media (DMEM, 10% FBS, 1% Pen-Strep, and 0.5% fungizone) at 37°C and 5% CO2. After 24 h of incubation, the wound was made by scraping the cells in the well using a 10–200 L sterile pipette tip. Cell wells were washed with PBS. Cells were incubated with *A. vera* extract with various concentrations (500, 250, 125, 50, and 5 μg/mL), DMEM media as a control group, and Algisite-M® as a positive control. The lacerations were photographed at 0 h using a camera Moticam 350, 10× in JPEG format. The next series of images was photographed after 24 and 48 h of incubation. Migration rates were analyzed using "ImageJ" software, comparing the percentage of covered area (24 and 48 h) with the values obtained at 0 h. This experiment was carried out in triplicate.

Statistical Analysis

The data were analyzed using SPSS 22 software for Windows. All data are analyzed using one-way ANOVA test. The results obtained were compared with the control group with the multiple comparison *post hoc* LSD test. Differences between groups were significant with $p < 0.05$.

Results

Fibroblasts 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

The effect of multiple doses of *A. vera* on cell survival could be assessed by the viability of fibroblasts given short-term exposure of 24 h (Figure 1). Administration of large doses of *A. vera* to small doses gradually increases the viability of fibroblasts. The highest increase in viability was observed at a dose of 5 μg/mL. All *A. vera* concentrations showed a statistically significant difference in increasing viability compared to the control group ($p < 0.05$).

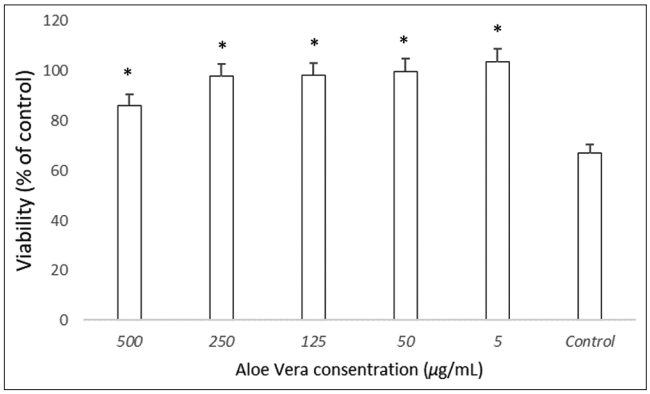

Figure 1: Cell viability after 24 h of treatment with various doses of Aloe vera. There was a difference in the viability of A. vera compared to the control ($p < 0.05$). Analysis of three trials ($n = 3$)

Effect of A. vera on fibroblast cell morphology

Healthy fibroblast cells can be observed by looking at the cell morphology. Normal fibroblast cells show an elongated, ovoid, and multipolar shape. Cell morphology changes did not occur after being given *A. vera* at 24 h (Figure 2). These results indicate that *A. vera* does not cause toxic effects on fibroblast cells.

Effect of A. vera on fibroblast cell viability

Cell viability at 24 and 48 h was measured to determine the effect of long-term exposure to *A. vera* on fibroblast cells. The administration of *A. vera* increased cell viability along with the administration of the smallest dose at 24 h. There was a decrease in cell viability after 48 h of intervention (Figure 3). A dose of 5 μg/mL had the highest viability value compared to all *A. vera* groups and was significantly different ($p < 0.05$). The dose of 500 μg/mL showed the lowest proliferation value, statistically significant compared to the 5 μg/mL group and control cells.

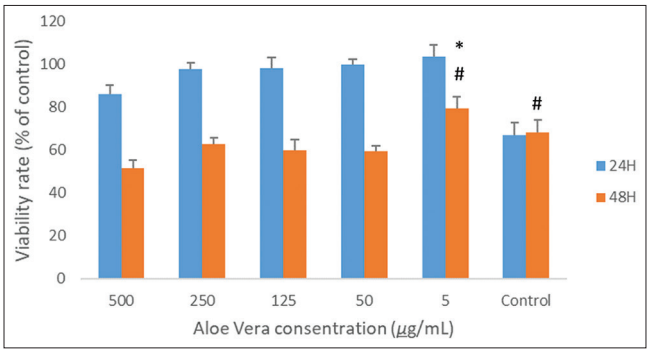

*Figure 3: Viability of fibroblast cells at 24 h and 48 h after being given Aloe vera. ANOVA test in 24 h ($p = 0.000$) and 48 h ($p = 0.009$). LSD post hoc; * < 0.05 compared to all A. vera groups; # < 0.05 compared to A. vera group at a dose of 5 μg/mL*

Fibroblast in vitro wound scratch assay

At the start of the *in vitro* wound scratch assay, there were no cells in the wound area (Figure 4). Migration of fibroblast cells increased after 24 h of *A. vera* treatment. Fibroblast cells begin to migrate to the center of the wound. Administration of *A. vera* in doses of 250, 125, 50, and 5 μg/mL significantly increased migration after 24 h of treatment compared to the 500 μg/mL and control groups (Figure 5). Migration of fibroblast cells filled the wound area after 48 h of intervention. *A. vera* doses of 500, 125, 50, and 5 μg/mL significantly increased migration after 48 h of

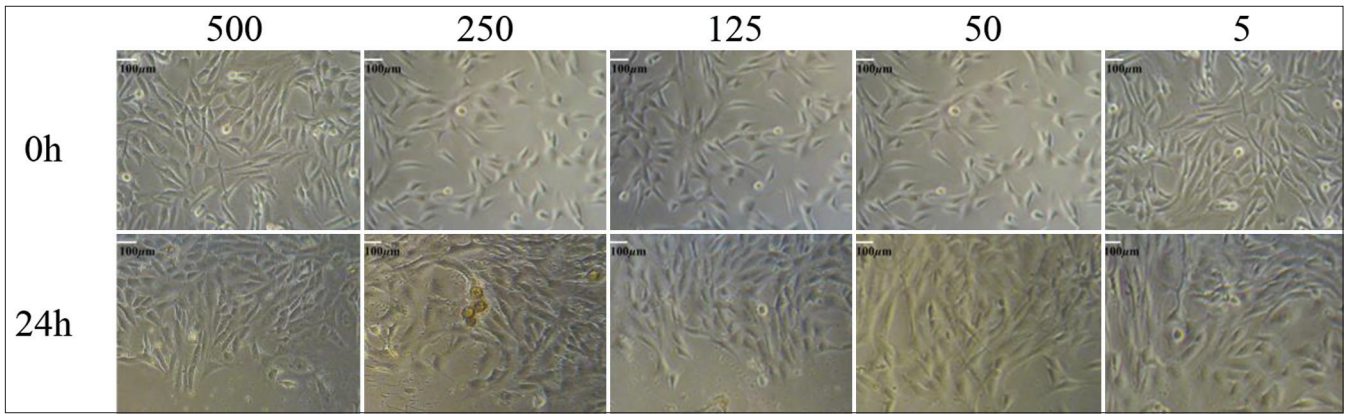

Figure 2: Morphology of fibroblast cells after being given Aloe vera. Images of fibroblasts were taken with 10× using a camera Moticam 350 microscope. Incubation of A. vera did not change cell morphology

treatment compared to the 250 μg/mL dose group and the control group.

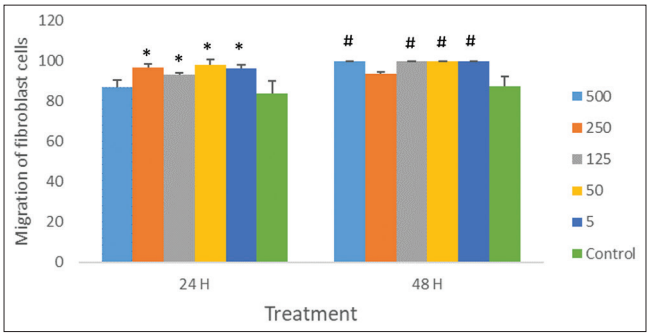

*Figure 4: Migration of fibroblast cells given Aloe vera after 24 and 48 h. ANOVA test in 24 h (p= 0.000) and 48 h (p= 0.000). LSD post hoc; 24 h *(p < 0.05) compared to the 500 μg/mL group and control cells; 48 h # (p < 0.05) compared to the 250 μg/mL group and control cells*

Discussion

The development of herbal medicine has attracted worldwide interest in recent years. Herbs are used because of their availability, lower costs, and lower side effects [23]. Some herbs have the potential to heal wounds without damaging healthy tissue, reduce infection, and increase wound healing time. We examined local Indonesian *A. vera* to determine the proliferation and migration of fibroblast cells. This shows that *A. vera* significantly increases cell viability and migration after the intervention.

Measurement of cell viability and proliferation is essential during the wound healing process. MTT test is a technique for estimating cell viability by measuring the metabolic activity of cells. Giving *A. vera* 24 h showed an increase in viability depending on the dose given. The rise in fibroblast cell viability was maximal at the lowest dose of 5 μg/mL. After 48 h, there was a decrease in cell viability. Different results were revealed by Soba *et al.* that 48 h of *A. vera* administration still increased viability. Samira *et al.* also reported an increase after 48 h of *A. vera* treatment, but it was not significantly different from the control group [18]. Other results can occur due to the use of *A. vera* from different types and locations.

Observation of cell morphology after being given *A. vera* did not appear to be abnormal. The morphology of healthy fibroblasts is elongated and multipolar. In their research, Saba *et al.* [17] showed that there were changes in fibroblasts that were flat and fan shaped, but this morphology was a characteristic of cells that had migrated. Fibroblasts will migrate to the wound site 48–72 h after injury and play an essential role in the wound healing [5].

We found that the administration of *A. vera* increased cell migration depending on the dose administered. *A. vera* group saw cell migration activity starting to move to the center of the wound after 24 h of intervention. Scratch wounds almost closed in the 50 μg/mL and 5 μg/mL concentration groups. At 48 h, it was seen that some of the scratches had closed in some *A. vera* groups, except for the 250 μg/mL concentration group which had not closed the scratches compared to the control group. These results are also supported by the previous studies where *A. vera* can increase the proliferation and migration of rat dermal fibroblast cells [18]. Teplicy *et al.* [24] also confirmed that the benefits of *A. vera* could increase the proliferation and migration of keratinocyte and fibroblast cells *in vitro*.

A. vera is believed to have pharmacological benefits such as antioxidants, antimicrobials, and good immunity for wound healing [25]. *A. vera* has

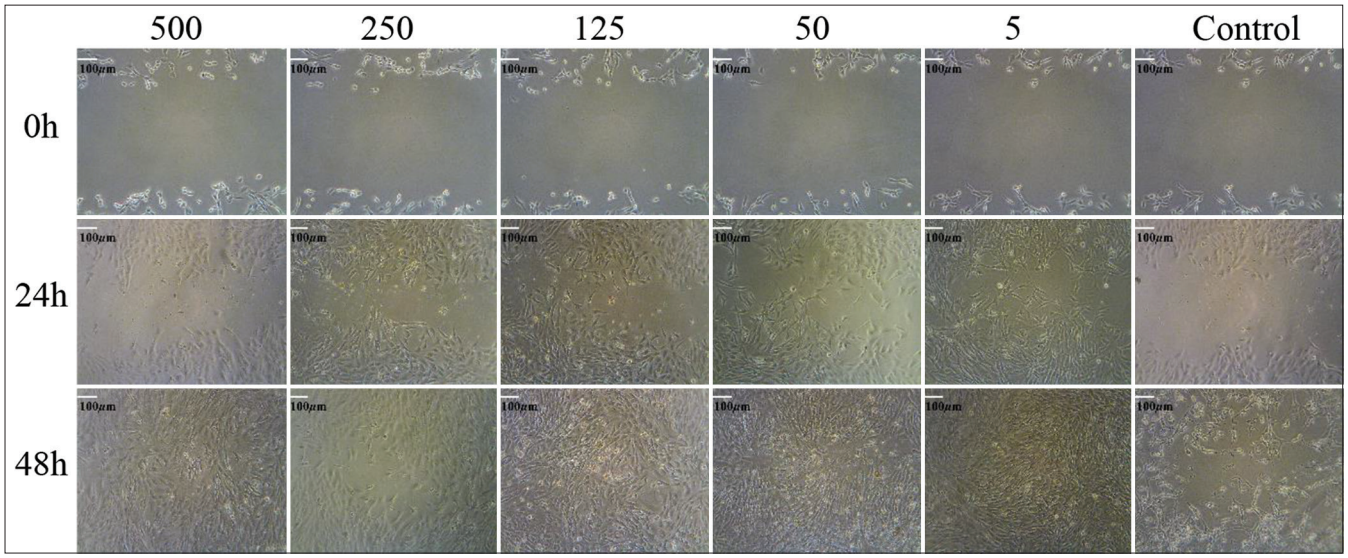

Figure 5: Migration of fibroblast cells starting from the beginning of the in vitro wound scratch assay and 24 h, 48 h after intervention with Aloe vera. The scale bar uses a 100 μm magnification viewed with a 10× microscope

components such as 5.5 kDa glycoprotein, which increases growth factor TGF-β1, and VEGF, one of the essential factors for tissue and organ growth [26], [27]. According to Wahedi and Hussain Mustatab *et al.*, giving *A. vera* can shorten healing time by increasing angiogenesis through activation of SMAD and MAPK [28]. Angiogenesis plays a role in forming new blood vessels from the last blood vessels, which are regulated by signals from the surrounding serum and ECM. The polysaccharide content in *A. vera* can induce the expression of MMP-3 and metallopeptidase inhibitor-2 genes, which directly regulate wound healing activity *in vivo* [29].

In general, the effect of *A. vera* can increase cell proliferation and migration. Different findings occur due to plants from different locations with compositions and isolation techniques to extract *A. vera* [17]. According to Esua *et al.* [30], *A. vera* 's biological activity can be antagonistic and protagonist depending on veracylglucan B and C. The content of veracylglucan B in high amounts will cause delays in wound healing, while veracylglucan C in high amounts will result in poor wound healing positive [30]. Therefore, local Indonesian *A. vera* still needs to be explored *in vivo* and *in vitro* to accelerate wound healing.

Conclusions

The lowest dose of *A. vera* extract had a beneficial effect on wound healing. It increases the viability and migration of fibroblast cells. *A. vera* did not change the morphology of fibroblast cells.

Ethical Approval

This study was approved by the Integrated Research and Testing Laboratory (LPPT), Universitas Gadjah Mada, Indonesia, with the ethical expediency number 00012/04/LPPT/III/2020.

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