The Role of Fibroblast Proliferation in Wound Healing by Different Plants: An Experimental Study

Marisa Riliani1 a, Indra Kusuma2 b, Abdul Halim3 c, Aliya Muhammad3 d, Akbar Fitrianto3 e, Ida Bagus Eka Narendra3 f

1Department of Anatomy, Faculty of Medicine, Universitas Yarsi, Jakarta, Indonesia
2Department of Physiology, Faculty of Medicine, Universitas Yarsi, Jakarta, Indonesia
3Faculty of Medicine, Universitas Yarsi, Jakarta, Indonesia

Keywords: Manihot esculenta, Ageratum conyzoides L., Fibroblast, Wound Healing

Abstract: Wound healing is a process that consisted of inflammation, proliferation, and migration of fibroblasts. Indigenous people commonly used Manihot esculenta and Ageratum conyzoides L. as bleeding wounds therapy. This study aimed to investigate these two plant extracts on fibroblast proliferation and migration capabilities. Skin fibroblasts were cultured with a medium conditioned for each extract with different concentrations (0.5%, 1%, 2%, 4%), positive and negative control groups. In evaluating proliferation, fibroblasts were incubated by CCK-8 and measured by spectrophotometer expressed as optical density (OD). The evaluation of migration was visualized by scratch assay. The distance between each edge of the scratch was measured using T-scratch software and expressed as the area's closure percentage. There were significant differences in fibroblast proliferation rate in the groups receiving 0.5% Manihot esculenta (p=0.00) and 2% Ageratum conyzoides L. (p=0.00). The migration of fibroblasts was no different, with 0.5% Manihot esculenta (p=0.40) and 2% Ageratum conyzoides L. (p=0.18). Manihot esculenta and Ageratum conyzoides L. could be considered to be used as agents to accelerate wound healing by increasing the fibroblast proliferation. Our findings suggest in vivo studies for tissue regeneration.

1 INTRODUCTION

In 2013, skin wound affected 8.2% population in Indonesia. Metabolic diseases and infections can influence the healing time; it could make a slow or even non-wound healing process. Slow or non-healing wounds affect millions of people worldwide and result in enormous health care expenditures. The skin is our outer part of the body, which can protect us from many environmental stresses. Injured skin sets into motion a series of repair mechanisms directed to the injured tissue. Wound healing is a skin reparative process consist of inflammation, proliferation, and migration of fibroblasts. The role of fibroblasts is to synthesize and integrate protein and elastin inside the extracellular matrix from some large part of the mesenchyme tissue (Simon, 2020).

Along with proper wound care products, it works together to repair and replace devitalized tissue. Many topical drugs are used to create and maintain a moist environment and provide healing conditions (Lordani et al., 2018). They are often expensive, and drugs price increased up to 15% every year (Blumberg, 2019). Therefore, we need to find alternative ingredients that easy to find, at a lower cost and have a shorter healing time. Recently, so many plants are coming out as tools for therapeutic application.

For decades, indigenous people have commonly used the leaves of Manihot esculenta and Ageratum

https://orcid.org/0000-0002-5025-793
https://orcid.org/0000-0001-9350-431X
https://orcid.org/0000-0003-1842-7646
https://orcid.org/0000-0002-9249-6044
https://orcid.org/0000-0002-8553-1550
https://orcid.org/0000-0001-7800-1335
conyzoides L. as therapeutic applications for bleeding wounds (Oktaviani et al., 2019). In in vivo study, Ageratum conyzoides L. increased cellular proliferation and collagen synthesis (Arulprakash et al., 2011). Meanwhile, Manihot esculenta increased the gingival wound healing process (Nisa et al., 2013). Nevertheless, these plants' efficacy has never been found to fibroblast cells, which is very important for the healing process.

Therefore, in this study, we aimed at investigating fibroblast proliferation and migration capabilities in vitro in the presence of ethanol extracts of Manihot esculenta and Ageratum conyzoides L.

2 MATERIALS AND METHODS

2.1 Research Design

The cells were cultured with a medium conditioned for each extract with different concentrations (0.5%, 1%, 2%, 4%), positive and negative control groups.

For migration assay, cell culture was divided into four groups; two groups received the most significantly induced fibroblast proliferation for each extract: 0.5% Manihot esculenta and 2% Ageratum conyzoides L., two other groups as positive and negative control groups.

2.1.1 Time and Place

The experiment was conducted from October to December 2019 at Laboratorium Penelitian Terpadu Universitas Yarsi.

2.1.2 Populations and Samples

The preputium skin fibroblast from Biorepository of Universitas YARSI was routinely cultured in Dulbecco's modified Eagle's medium (DMEM), low glucose supplemented with 2 mM L-glutamine and 10% fetal bovine serum (FBS). All cells were grown in 100 units/mL penicillin, 100 µg/mL streptomycin, and 0.25 µg/mL amphotericin B. Cells for the experiments were used at passage 3.

Plant materials of Manihot esculenta and Ageratum conyzoides L. were collected in October 2019 in the Caringin village, west java. The leaves were dried at room temperature for five days and then cut into fragments of approximately 5 cm. The fragments were soaked in 70% ethanol and filtered. A rotatory evaporation machine evaporated the filtrate.

2.1.3 Viability and Proliferation Assay

Fibroblasts were seeded at the concentration of 3000 cells/well in a 96 well plate and allowed to incubate overnight. After the cells attached to the well surface, cells were washed in phosphate-buffered saline (PBS). The cells were cultured with a medium conditioned for each extract with different concentrations (0.5%, 1%, 2%, 4%), positive and negative control groups for 48hr. The culture medium was discarded every indicated time points and added 10 µl CCK-8 and 90 µL PBS. After 90 minutes of incubation with CCK-8, absorbance detected at 450 nm using a Tecan microplate reader. The results expressed in optical density (OD) units as compared to untreated cells.

2.1.4 Migration Assay

Fibroblasts were seeded at a concentration of 40.000 cells/well in a 12 well plate and incubated until confluence. A scratch was made using a 10 µl pipette tip for each well. After removed the media, the cells were washed in PBS and then added medium conditioned with 0.5% Manihot esculenta and 2% Ageratum conyzoides L. The distance between scratch areas was measured by optical microscopy every two hours for 48 hours using Nikon advanced research elements 3.21.00 software and expressed as a percentage of the area's closure compared to untreated cells.

2.1.5 Data Analysis

Three technical replicates performed the experiments for each treatment. All statistical analyses were performed by comparing 0.5% Manihot esculenta and 2% Ageratum conyzoides L using unpaired Student's t-test.

3 RESULTS

3.1 Cell Viability and Proliferation

At 48 hours, the extracts of 2% Ageratum conyzoides L. was the most significantly induced fibroblast proliferation at 0.22 ± 0.02 (Figure 1) and showed intact fibroblast morphology (Figure 3, panel D). The extracts of 0.5% Manihot esculenta was the most significantly induced fibroblast proliferation at 0.16 ± 0.01 (Figure 2) and showed intact fibroblast morphology (Figure 4, panel B). DMSO significantly decreased fibroblast proliferation at 0.01 ± 0.00 and
showed damage to fibroblast morphology. The control group indicated fibroblast proliferation at 0.07 ± 0.01 and showed intact fibroblast morphology. There were significant differences in fibroblast proliferation rate in 2% Ageratum conyzoides L. (p=0.00) and 0.5% Manihot esculenta (p=0.00) compared to the control group.

Table 1: T-test for fibroblast proliferation in different concentrations of Ageratum conyzoides L. at 48 hours.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Ageratum conyzoides L</th>
<th>T-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5%</td>
<td>0.15</td>
<td>0.00</td>
</tr>
<tr>
<td>1%</td>
<td>0.19</td>
<td>0.00</td>
</tr>
<tr>
<td>2%</td>
<td>0.22</td>
<td>0.00</td>
</tr>
<tr>
<td>4%</td>
<td>0.18</td>
<td>0.01</td>
</tr>
<tr>
<td>DMSO</td>
<td>0.01</td>
<td>0.00</td>
</tr>
<tr>
<td>Control</td>
<td>0.07</td>
<td></td>
</tr>
</tbody>
</table>

Data were expressed as optical density (OD)

Figure 2: Fibroblast viability and proliferation in different concentrations of Ageratum conyzoides L. at 48 hours.

Figure 3: Fibroblast Morphology in different concentration of Ageratum conyzoides L. at 48 hour. (A) control group, (B) 0.5%, (C) 1%, (D) 2%, and (E) 4% extract in complete medium, (F) 10% DMSO.

Table 3: Migration of fibroblast.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Ageratum conyzoides L</th>
<th>0.5% Manihot esculenta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>61.42 ± 7.88</td>
<td>66.65 ± 4.17</td>
</tr>
<tr>
<td>2%</td>
<td>64.35 ± 6.25</td>
<td>35.36 ± 11.21</td>
</tr>
<tr>
<td>0.5% Manihot esculenta</td>
<td>31.08 ± 12.29</td>
<td>38.28 ± 8.89</td>
</tr>
<tr>
<td>DMSO</td>
<td>0.60 ± 0.15</td>
<td>0.43 ± 0.2</td>
</tr>
<tr>
<td>Control</td>
<td>2.07 ± 2.86</td>
<td>0.43 ± 0.2</td>
</tr>
</tbody>
</table>

3.2 Cell Migration

At 48 hours, the wound was almost closed for each group. However, there were no significant differences in fibroblast migration rate in 2% Ageratum conyzoides L. (p=0.18) and 0.5% Manihot esculenta (p=0.40) compared to the control group.

Data were expressed as wound closure percentage (%). At 48 hours, 2% Ageratum conyzoides L at 2.07 ± 2.86, and 0.5% Manihot esculenta at 0.43 ± 0.2 .
4 DISCUSSION

Wound healing time can take a year or more to finish. Some wounds do not heal in a timely and orderly manner. It is disturbed by various factors such as infection, tissue hypoxia, necrosis, exudate, and excess inflammatory cytokines. The wound healing process has four overlapping stages: homeostasis, inflammation, proliferation, and migration (Addis et al., 2020). While platelets have a role in clot formation during homeostasis, inflammation cells debride injured tissue during the inflammation phase. At the proliferative phase, occur epithelialization, fibroplasia, and angiogenesis (DesJardins et al., 2018).

Meanwhile, granulation tissue forms and the wound begins to contract. In the maturation phase, collagen forms tight cross-links to other collagen and with protein molecules. Fibroblasts have a crucial role in all of these phases, including the deposition of extracellular matrix (ECM) components, wound contraction, and new ECM remodeling (Sumbayak, 2016).

Plants and herbs are active medicinal, are used to stimulate stem cell proliferation, regeneration, and rehabilitation in damaged tissue (Cragg and Newman, 2017). Several studies found that the leaves' active ingredients mainly activate stem cell regeneration potential (Maioli et al., 2010). Plant extracts or plant-derived compounds are preferred because of fewer side effects and widespread availability (Agyare et al., 2014). Moreover, wound healing management can be elicited by the antioxidant activity of some plant extracts (Süntar et al., 2012).

The antioxidant flavonoid of Ageratum conyzoides L. has anti-inflammatory effects, especially quercetin, that can inhibit β-glucuronidase, decrease leukotriene, inhibit histamine, inhibit some enzymes such as ATPase, phosphodiesterase, and protein kinases (Galati, 2008). Meanwhile, the extract of Manihot esculenta has an anti-inflammatory effect since having gallic acid, vitamin C, flavonoid, saponin, tannin, and triterpenoid as antioxidant compounds (Oktaviani et al., 2019).

Reactive oxygen species are involved in many infections, degenerative diseases, cancer, and even wound healing. Antioxidants enhance the healing of wounds by reducing the damage caused by oxygen radicals. Plant-derived antioxidants benefit from their redox properties, which allow them to act as hydrogen donors, reducing agents, hydroxyl radicals (OH), or superoxide radicals (O2) scavengers (Geethalakshmi et al., 2013).

These two extracts have toxic components that may interfere with the healing process since the data showed no significant difference in fibroblast migration. Ageratum conyzoides L. contains pyrrolizidine alkaloids (PAs), which have been reported to be hepatotoxic, mutagenic, and carcinogenic (Bosi et al., 2013). Manihot esculenta contains toxic agents cyanogenic glycosides, made up of 95% linamarin and 5% lotaustralin (Faezah et al., 2016).

Pyrrolizidine alkaloids are widely distributed in plants throughout the world. Alkaloids present a lipophilic character, soluble in apolar organic solvents and alcohol. Pyrrolizidine alkaloids can penetrate the nucleus and react with DNA, causing DNA cross-link and DNA-protein cross-link to elicit an abnormal function, which will cause damage (Moreira et al., 2018).

Cyanogenic glycosides are bioactive plant products derived from amino acids—the primary biological function is as a plant defense system against the effects of distinct animals. Acute poisoning of animals and humans from cyanogenic consumption can induce rapid and drastic inhibition of the respiration system in mitochondria (Vetter, 2017).

Our data showed that the extracts could enhance wound healing by stimulating the proliferation of fibroblast. Within this context, the extracts of Ageratum conyzoides L. and Manihot esculenta could be used in the future as a topical therapeutic application to stimulate the wound healing process and antioxidant responses in damaged skin.
5 CONCLUSION

The study of Ageratum conyzoides L and Manihot esculenta extract’s leaves proved it could accelerate wound healing. The extracts increase fibroblast proliferation in vitro. Further in vivo study is needed for tissue regeneration applications.

ACKNOWLEDGEMENTS

We want to thank Herbal Laboratory-Universitas Yarsi and Yarsi University Foundation for the support.

REFERENCES


