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ETHANOL EXTRACT OF *Abrus precatorius* (Linn) LEAVES INDUCE PROLIFERATION OF HUMAN FIBROBLASTS DERMAL IN VITRO

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ABSTRACT
Dermal fibroblasts are supporting cells that have an essential role in wound healing and new tissue growth. Abrus precatorius (Linn) (AP) is known as the saga plant, containing terpenoids, alkaloids, and flavonoids. This plant is proven to have an anti-inflammatory effect and wound-healing therapy empirically based. The study was to assess the ethanol extract of AP effect on dermal fibroblasts proliferation. Dermal fibroblasts were cultured on 96 wells plates in a complete DMEM medium containing 15, 30, 45, 50, 75, and 90 µg/mL of ethanol extract of AP for 48 hours. The cell viability was measured at two-times points (24 and 48 hours) using a cell counting kit-8 (CCK-8) and read at 450 nm. Cell viability decreased significantly in the fibroblast group that received AP ethanol extract at concentrations of 45, 50, 75, and 90 µg/mL compared to the control group without treatment and solvent control at both 24 and 48 hours of observation ($p<0.05$). Otherwise, the viability of the cell that received the extract at concentrations of 15 and 30 µg/mL was higher than that of the solvent control group ($p<0.05$) at the two-times point. The ethanol extract of AP leaves potentially boosted the proliferation of dermal fibroblast cells at low concentrations but less cell viability at higher doses.

Keywords:
fibroblast, Abrus precatorius, proliferation, cell viability

INTRODUCTION

Fibroblasts are the preeminent cells in the dermis which play an essential role in maintaining the integrity of the supporting tissue structure. These cells synthesize and reconstruct extracellular matrix proteins such as collagen, reticulum, elastin, glycosaminoglycans, and glycoproteins. These proteins influence cell proliferation and survival, cell communication, adhesion, differentiation, migration, autophagy, angiogenesis, and regulation of immunity. (Stunova & Vistejnova, 2018; Karamanos et al., 2021).

Dermal fibroblasts are crucial component of wound healing. Platelets and macrophages respond to inflammation by secreting and releasing chemoattractants in the form of platelet-derived growth factor (PDGF), interleukin-1, beta (IL-1β), and tumor necrosis factor-alpha (TNF-α). These chemoattractants attract fibroblasts to migrate to the wound site within 24-48 hours after injury. Fibroblasts produce an extracellular matrix to reduce and replace the fibrin clot. This matrix complex supports and regulates further

migration and activity of fibroblasts, as well as activating signals for angiogenesis, granulation tissue, and epithelialization. (Bainbridge, 2013)

The *Abrus precatorius* L. (AP) in Indonesia is known as the saga plant. It has been used empirically in traditional medicine for wound healing, cancer sores, sore throats, coughs, bronchitis, jaundice, and malaria. The AP leaves have pharmacological effects in vitro and in vivo as antitumor, antimicrobial, insecticidal, antiprotozoal, antiparasitic, anti-inflammatory, antioxidant, immunomodulatory, antifertility, and antidiabetic. (Huiqin et al., 2022)

The AP leaves contain pinitol, triterpene glycosides, glycyrrhizin, and alkaloids such as hepaphotine, precatorine, abrine, choline. Triterpene glycosides consist of abusosides A, B, C. Other active compounds are abruslacton A, abrusgenic acid tritepen, methyl abrusgenat, liquirtiginin-7-diglycoside, liquirtiginin-7-monoglycoside, toxifolin-3-glucoside, and the flavonoid vitexin 2. (Aswin et al., 2022).

Based on the active compound content of AP and its anti-inflammatory potential, research is needed to assess the effect of AP on wound healing. Our initial research focused on the influence of ethanol extract of saga leaves on dermal fibroblast cell proliferation.

METHOD

Ethanol extract of AP leaves

Ethanol extract AP leaves was prepared by drying 100 grams of fresh leaves. The leaves were softened using a blender, then macerated in 96% ethanol solvent in a closed container, and protected from sunlight for two days. After that, the mixture was filtered to obtain macerate. The dregs were macerated in 96% ethanol using the same procedure. Maceration was carried out until translucent macerate was obtained. Macerate evaporated using an evaporator at a temperature of 400C.

The extract dissolved in Eagle-modified Dulbecco medium (DMEM; Gibco, Grand Island, NY, USA) with 0.1% dimethyl sulfoxide (DMSO) addition. DMSO is useful for facilitating the penetration of extracts into cells.

Cell Culture

Human dermal fibroblast (HDF) cells obtained from cryo-stock of Biorepository of Stem Cells Research Center Universitas YARSI, Indonesia. Cells were grown in complete medium which containing DMEM supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic (penicillin-streptomycin-amphotericin B; Gibco, Grand Island, NY, USA) in an incubator with a temperature of 37oC and 5% CO2.

Cells were seeded into 96-well culture plates (TRP, Switzerland, Europe) at a density of 5 × 103 cells per well in a complete medium with three repetitions at each treatment group. Twenty-four hours after sufficient cell number was reached, the cells were treated with different AP concentrations ranging from 15, 30, 45, 60, 75 and 90 ug/mL for 96 hours to assess cellular viability and toxicity. As the control group were no treatment control (NTC) cell and the solvent control (SC) cell that was treated with 0.1% DMSO. All treatments were repeated 2 times.

HDF cell viability was evaluated at three-time points (24, 48 and 96 hours), using CCK-8 (cell counting kit-8 (2-(2-methoxy-4-nitrophenyl)-3- (4-nitrophenyl)- 5- (2,4-disulfophenyl)-2H-tetrazolium, monosodium salt; SIGMA) and read using a spectrophotometer at 450 nm.

CCK-8 assay

After 24 hours of incubation, the culture media was removed entirely and replaced with 100 uL of CCK-8 solution (10 uL) at a 1:10 dilution with complete medium followed by

incubation for 60 minutes at 37oC, 5% CO2 (0 hours). Subsequent measurements were taken 24 48 and 96 hours after administration of AP.

Statistic

Statistical analysis was done by one-way ANOVA and post hoc test by Least Significant Difference (LSD). A value of $P < 0.05$ was considered statistically significant.

RESULT AND DISCUSSION

Figure 1 shows the morphology of fibroblasts in their fusiform shape. The cells used in this study were cells in passage six. The proliferation assay carried out based on the principle of the tetrazolium salt WST-8 {2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disuLfophenyl)-2H-tetrazolium} in the CCK8 kit will be reduced by the living cell dehydrogenase enzyme to produce an orange color that is proportional to the number of viable cells.

Figure 1. Morphology of Human Fibroblast Dermal
Source: Stem Cells Research Center Universitas YARSI

Figure 2 demonstrates fibroblast viability after exposure to AP ethanol extract (EAP) in six different concentrations. Cells observed at 24, 48 and 96 hours. Cell viability in the 15 and 30 ug/mL concentration groups was not significantly different from those of the control group without treatment. Furthermore, the number of cells in the two concentration groups significantly increased compare with the 0.1% DMSO solvent control group ($p<0.05$). On the other hand, EAP at concentrations of 45, 60, 75 and 90 had a toxic effect as indicated by the lower absorbance value that was linear with the dose. These results are consistent at three observation points. These implies that EAP at concentrations of 15 and 30 ug/mL is not toxic and can overcome DMSO toxicity.

Observations with a microscope exhibited changes in cell morphology to become irregular and changes in cell density in line with increasing concentration and duration of EAP exposure. Figures 3, 4, and 5 respectively represent the morphological appearance of fibroblasts after exposure to various AP concentrations for the three exposure durations.

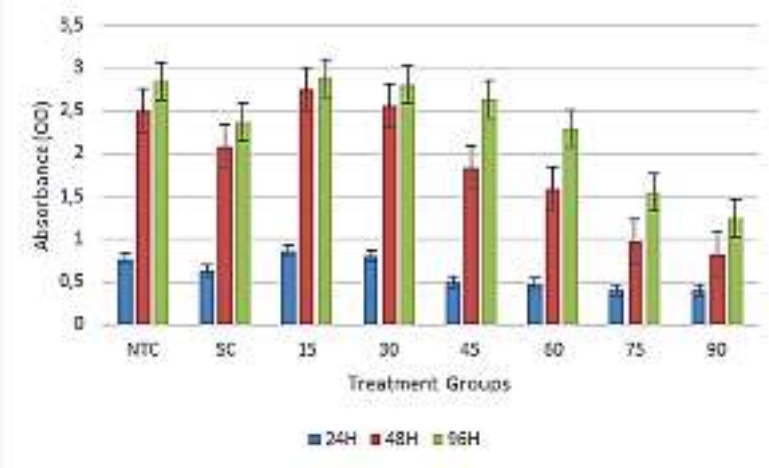

Figure 2. Effect of various AP concentrations on HDF cell viability at 24,48 and 96 hours of examination

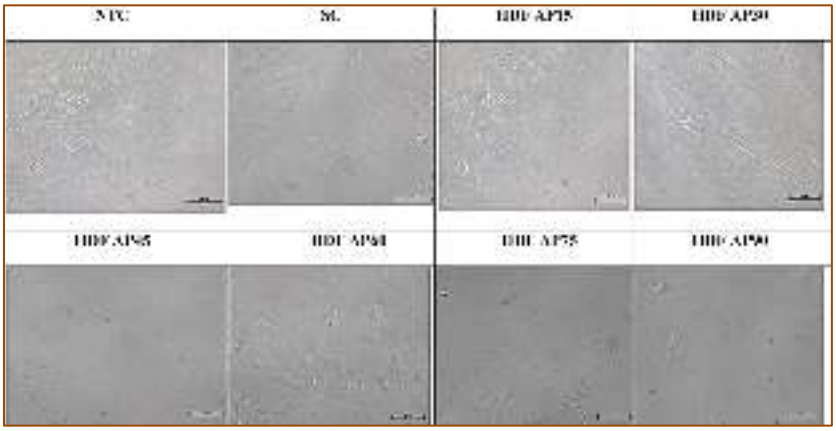

Figure 3 Viability of HDF 24 hours after exposure to different concentrations of AP

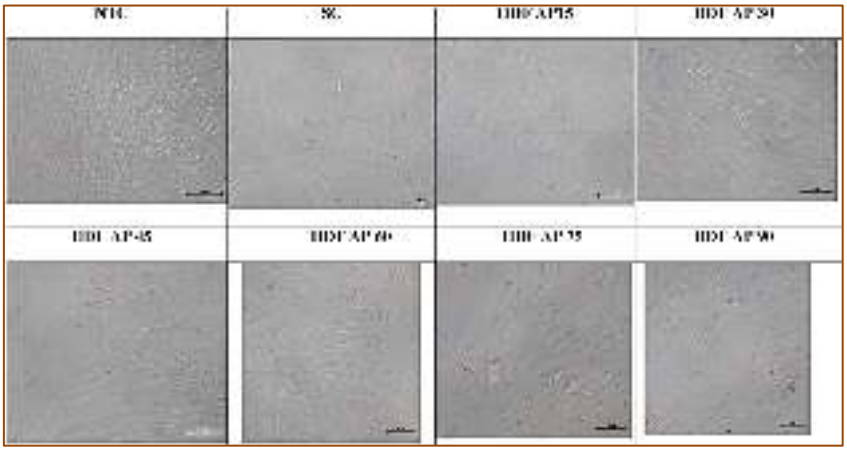

Figure 4 Viability of HDF 48 hours after exposure to different concentrations of AP

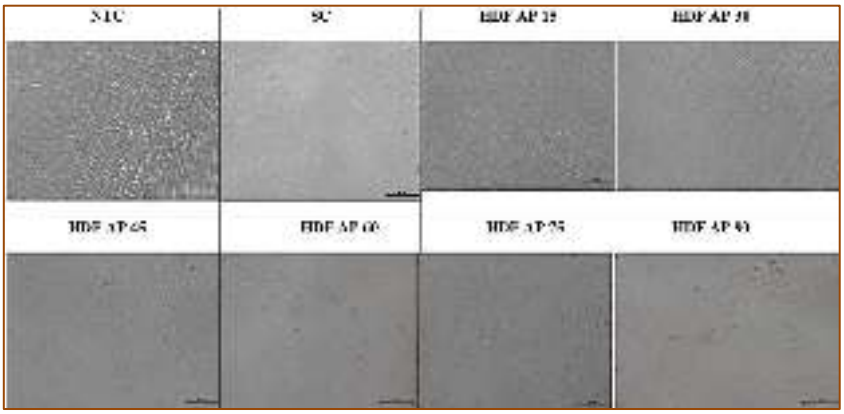

Figure 5 Viability of HDF 96 hours after exposure to different concentrations of AP

DMSO is an organic solvent that can dissolve both polar and non-polar substances. This solvent can increase drug penetration into cell membranes, so it is often used in laboratory studies for compounds those low solubility in water. DMSO is also frequently used as a cell line cryopreservation agent. (Villagran et al., 2022). However, DMSO is known to be toxic to cell cultures in vitro. This toxicity depends on the concentration and duration of exposure to DMSO, in conjunction with the type of cells used. The Study reports DMSO did not affect the viability of human peripheral blood mononuclear cells (PBMC) at concentrations up to 10% within 1 hour of exposure. Other studies have stated that DMSO at a dose higher than 1% was toxic to most mammalian cells when exposed in vitro. This solvent at 0.1% concentration decreased cell proliferation which referred to cell cycle, differentiation, or cell death. (Sigh et al., 2017). DMSO as a fibroblast cryopreservation agent at a concentration of 1% reduced cell viability by up to 73%, while DMSO at a concentration of 0.5% viability of fibroblasts was more than 80-%. (Costa, et al., 2017).

In this study, the control cell group exposed to 0.1% DMSO showed a 5-10% reduction in cell viability compared to the control group without treatment. All cell groups that received EAP were also exposed to 0.1% DMSO as a solvent for the extract. However, different concentrations show different results. Cell viability in the group that received EAP concentrations of 15 and 30 ug/mL was higher than the solvent control group and the group with other EAP concentrations. It is estimated that first; The EAP at 15-30 ug/mL can neutralize DMSO toxicity, and second; EAP doses greater than 30 ug/mL are toxic in themselves beyond the toxicity of DMSO.

As previously mentioned, AP leaves are rich in antioxidants. Research reports antioxidants induce cell proliferation in vitro. Asiaticoside, an active substance from the Centella asiatica plant, which has an antioxidant effect is reported to increase the proliferation of dermal fibroblast cells. (Yulianti, et al., 2015). Phenolic compounds from various plants were reported to reduce oxidative stress and increase fibroblast cell proliferation. (Sawdoska et al., 2021). In addition, both natural and synthetic antioxidants were reported to significantly increase the extension of the replicative lifespan of fibroblasts. This effect is thought to be a direct and indirect antioxidant effect through the activation of signaling pathways. (Bartosz & Bartosz, 2020).

Antioxidants are known to have hormetic effects, namely providing a stimulating effect at low doses and an inhibitory effect at high doses. Antioxidant induces Human endometrial mesenchymal stem cells proliferation when applied to the quiescent cells but induces irreversible cell cycle arrest, DNA strand breaks accumulation, and DNA damage response activation when applied to the proliferating cells (Kornienko et al., 2019). Based on this information, it is suspected that the hormetic effect of EAP could explain the decrease in

fibroblast viability in the cell group that received EAP at a concentration of more than 30 ug/mL. Further research is needed to assess the mechanism of EAP toxicity at these doses.

CONCLUSION

The ethanol extract of AP leaves potentially boosted the proliferation of dermal fibroblast cells at low concentrations but less cell viability at higher doses.

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