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Chlorogenic acid in preventing and curing ultraviolet-induced damage in human skin fibroblast as an antiaging cell model

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ABSTRACT

Continuous ultraviolet (UV) irradiation stimulates the over-production of reactive oxygen species (ROS) to cause degenerative diseases. Chlorogenic acid (CA) is found as plants antioxidant that promises medicinal effects. This study examined CA protection against UV-damage in human skin fibroblast (BJ) cells both for curative and preventive therapy. BJ cells were exposed to UV radiation and the addition of CA (6.26-100 μg/mL) by preventive and curative addition methods. The cells viability analysis was conducted employing MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay. CA treatment before UV exposure exhibited an increased percentage of viability cells than the positive control. In detail, the series of CA concentration (6.25, 12.5, and 25 μg/mL) significantly enhanced the percentage of viable cells. The addition of CA after UV exposure denoted the same results. Furthermore, the lower CA concentrations used, the higher cell viability resulted. CA at dose 6.25 μg/mL showed the highest viability in cells, while CA 100 μg/mL resulted in the lowest viability. In short, CA can preserve and treat cells from UV exposure. The outcome suggested prevention and curative on UV-induced BJ cells, and the tested concentration is applicable for further experiments.

Keywords: aging, cell viability, chlorogenic acid, fibroblast cell, ultraviolet

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INTRODUCTION

Skin is the outer organ that is prone to a range of environmental influences, including ultraviolet (UV) penetration (Amaro-Ortiz et al., 2014). UV irradiation accounts for 5% of all solar radiation that reaches the earth's surface (Deng et al., 2018). UV irradiation generates photoaged-skin cancer (Bauernfeind et al., 2009; Geng et al., 2021). At 320-400 nm, UV penetrates deeper into the dermal layers. It is absorbed by skin chromophores to stimulates reactive oxygen species (ROS) production in dermal and extracellular matrix (Xuan et al., 2019). The oxidative damage contributes to the change in gene expression and DNA damage, all of which eventually lead to cell inflammation, photoaging, and photo-carcinogenesis (Gu et al., 2020; Ung et al., 2021). Besides, ROS can indirectly activate inflammatory pathways, impair genetic stability, cause apoptosis (Gabe et al., 2014), and ultimately interferes with matrix integrity and cause photoaging (Lee et al., 2012; Prunier et al., 2012).

Antioxidants play the role to neutralize ROS-induced damage (Dunaway et al., 2018). The imbalance between antioxidants and free radicals in the body induces oxidative stress, which damages biomolecules (Caleja et al., 2017; Phaniendra et al., 2015). The current study reported chlorogenic acid antioxidant effect (Girsang et al., 2019b). Chlorogenic acid is one dietary polyphenol that is widely found in natural extracts such as green coffee, tea (Meng et al., 2013), and snake fruit peel (Girsang et al., 2019a). CA has different subgroups that include caffeoylquinic, p-coumarylquinic, and feruloylquinic acids (Liang & Kitts, 2015).

CA also acts as antibacterial (Bajko et al., 2016) and anti-inflammation (Hwang et al., 2014). It up-regulates collagen (COL)-3 expression of the UV-aged fibroblast cells as the antiaging mechanism (Girsang et al., 2021). COL-3 is pre-procollagen that is important in wound healing (Kuivaniemi & Tromp, 2019). CA has significant functions that include therapeutic and preventative treatment for skin damage caused by UV exposure. The potential of CA as antiaging in UV exposure needed to be evaluated, especially in preventive and curative therapy. Various natural extracts or chemical compounds have been used to study the effects of UV exposure. Human skin fibroblast (BJ) cell is widely used and evaluated as an anti-aging model and can be employed in the examination of UV filter (Bruge et al., 2014).

Fibroblasts are the most basic connective tissue cells, synthesizing the basic material as well as fibers. These mesenchyme-derived cells also have a variety of roles, including recruiting immune cells in response to inflammation and tissue alteration during wound healing (Sahinturk et al., 2018). The morphology of BJ cells can be seen in Figure 1. Therefore, in the present study, UV-exposed BJ cells were used as an anti-aging model. To evaluate CA treatments, this study used viability assay using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay, a very competitive, rapid, sensitive, economical, and specific in vitro cytotoxicity assay (Ni et al., 2014). Accordingly, this study evaluated human fibroblast cell viability by CA treatment in preventive and curative therapy.

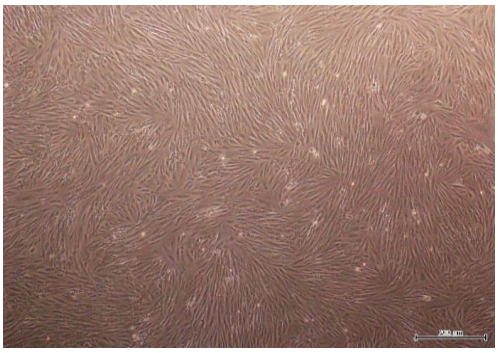

Figure 1. Morphology of fibroblast cell (Normal fibroblasts are large, flat, elongated cells (spindle-shaped). Cells at 80% density

MATERIALS AND METHOD

Materials

Chlorogenic acid (Chengdu, BP0345) and DMSO (Merck, 1029521000) were used for the treatment sample. Minimum Essential Medium (MEM, L0416-500; Biowest, Riverside, MO, USA), 10% fetal bovine serum (FBS, S1810-500; Biowest, Riverside, MO, USA), 1% antibiotic-antimycotic (ABAM, L0010-100; Biowest, Riverside, MO, USA), 1% Nanomycopulitine (L-X16-100; Biowest, Riverside, MO, USA), 1% amphotericin B (L0009-050; Biowest, Riverside, MO, USA), 1% L-glutamine solution (G8540; Sigma-Aldrich, MO 63103, USA) and 0,1% gentamicin (15750060; Gibco, Waltham, MA, USA) were used for growth medium of the cell. MTS assay (Promega, Madison, WI, USA) was used for the cell viability test.

Methods

Chlorogenic acid solution

Chlorogenic Acid 0,0002 g was soluble in DMSO and was used to make a total of 200 µg/mL CA solution. CA solution 200 µg/mL was divided in six concentrations are 3.13, 6.25, 12.5, 25, 50, and 100 µg/mL.

Cell culture

Human skin fibroblast (BJ) cell line (ATCC, CRL-2522) was provided by Aretha Medika Utama, Indonesia. Cells were grown in the mentioned medium at 37°C with 5% of CO_2 (Girsang et al., 2019c; Widowati et al., 2016).

Cell viability assay

The 80% confluent cells were detached and seeded as much as 5 x 10^3 in each well of 96-well plate. For preventive assay, CA was added a total of 20 µL for 24 h before UVA exposure for 75 mins at a dose of 300 J/cm^2 (Girsang et al., 2021). For curative assay, BJ cells were exposed to UVA light for 75 mins (300 J/cm^2), then added 20 µL CA at the given concentrations. BJ cells were grown in the incubator (5% CO_2 37°C) for 24 h (Girsang et al., 2021). 20 µL of MTS reagent was added into each well, then incubated for 4 h. The optical density was detected using Multiskan Go (Thermo Fisher Scientific, USA) at 490 nm (Widowati et al., 2016). Cell viability percentage for both preventive and curative assay were calculated with the Formula 1 and 2 below (Mughal et al., 2020):

$$\% \text{ cell viability} = \frac{(At-Ab)}{(Ac-Ab)} x\ 100 \quad (1)$$

$$\% \text{ cell inhibition} = 100 - \% \ cell\ viabilit \quad (2)$$

*At = Tested sample's absorbance (treated cells); Ab = Blank's absorbance (medium only); Ac = Control's absorbance (cells in medium)

Data Analysis

The statistical significance was obtained from Tukey HSD and Dunnet T3 post hoc tests (p-values < 0.05) in SPSS 20 software.

RESULT AND DISCUSSION

This work examined the impact of CA on the UV-exposed antiaging model in both curative and preventive therapy. The existence of ROS, such as H_2O_2, O_2^{*-}, and OH^*, challenges cellular signaling to damage skin (Huang et al., 2016). Both in preventive and curative therapies, the positive control group has lower viable BJ cells and higher inhibition compared to the negative control. These results imply that UV exposure causes skin cell damage. Similarly, a previous study reported that UV exposure affects the free radicals production known as ROS (Davalli et al., 2016). Aging skin fibroblast cells induced by UV increased ROS level (Girsang et al., 2021). ROS over-production is associated with inflammation

of skin fibroblast, indicated by a high level of Interleukin-1 beta (IL-1β), IL-6, Tumor Necrosis Factor-alpha (TNF- α) (Girsang et al., 2021).

Meanwhile, CA treatments, either after or before UV exposure, ameliorate cell damage. The current investigation demonstrates that the viability of BJ cells is increased by the addition of CA at 6.25, 12.5, and 25 g/mL, both as preventive and curative therapy in UV-induced fibroblast cells. Additionally, compared to the negative control, the cell viability is reduced by around 40–50% at CA concentrations of 100 and 50 µg/mL. CA simultaneously reduced the cell's viability and enhanced the inhibition. High concentrations of CA (100 and 50 µg/mL) were toxic toward UV-induced BJ cells, and this was in conformity with previous research that CA 100 and 50 µg/mL were toxic and damaged skin fibroblast (Girsang et al., 2019c).

Data in Figure 2A and Figure 2B demonstrate that preventive treatment using CA at 100 µg/mL generates the lowest cell viability and highest inhibition among groups. However, cell viability slightly increases, and cells inhibition noticeably decrease as the CA concentrations are lowered. At concentration of 12.5 µg/mL and 6.25 µg/mL, it significantly resulted in improved viability and lower inhibition than the positive control group.

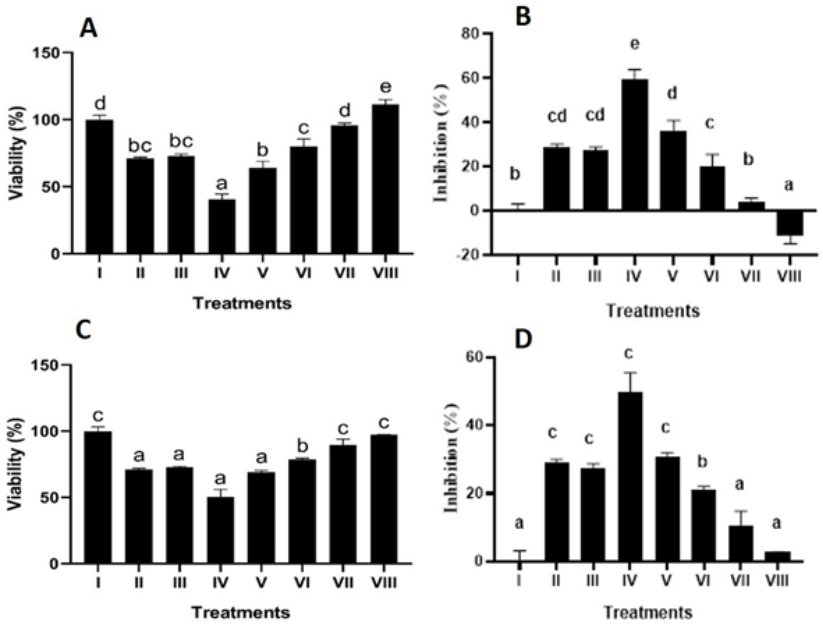

Figure 2. The effect of CA as preventive therapy (A-B) and curative therapy (C-D) on UV-induced BJ cells viability and inhibition

Note:
The data were in means ± standard deviation.
Each letter in Figure 2C and Figure 2D in Figure 2A (a, b, bc, c, and e) and Figure 2B (a, b, c, cd, d, e) shows significant differences among treatments with P<0.05 based on Tukey post hoc test.
Each letter (a, b, and c) shows significant differences among treatments with P<0.05 based on Dunnett T3 post hoc test.
*I: Negative Control; II: Positive Control; III: DMSO Positive Control; IV: Chlorogenic Acid 100 µg/mL; V: Chlorogenic Acid 50 µg/mL; VI: Chlorogenic Acid 25 µg/mL; VII: Chlorogenic Acid 12.5 µg/mL; VIII: Chlorogenic Acid 6.25 µg/mL.

A similar result is seen in curative therapy (Figure 2C and Figure 2D). Treatment with CA at 100 g/mL results in the lowest viability and highest inhibition among groups. Nevertheless, lower CA

concentrations result in higher viability and lower inhibition. CA 6.25 to 25 µg/mL significantly improved cell viability and was in line with previous studies that CA at the same concentrations increased cell viability in Pb-induced skin fibroblast cells (Girsang et al., 2019c).

In addition, both in preventive and curative therapies, CA 6.25, 25 µg/mL shows therapeutic effect in UV-induced skin fibroblast (Girsang et al., 2021). Previous studies reported CA 6.25, 25 µg/mL reduced ROS level and inflammatory mediators, including TNF-α, IL-1β in UV-aged fibroblast cells (Girsang et al., 2021) as well as reduced ROS level and TNF-α, IL-10 in Pb-induced fibroblast cells (Girsang et al., 2019c). Moreover, this data at concentration of 6.25 and 25 µg/mL, CA treatment in preventive application resulted in a higher therapeutic effect than curative application (Figure 2A and 2B). Likewise, the same treatment improved live cells, decreased cells death of the UV-induced skin fibroblast cells (Girsang et al., 2021). Additionally, CA 6.25, 25 µg/mL increased collagen type III gene expression, which is related to fibril integrity, is shown to be expressed more frequently when CA is present (Girsang et al., 2021). According to an in silico investigation, CA is an active component of the peel extract of the snake fruit (*Salacca zalacca* (Gaert.) Voss) to inhibit enzyme linked-skin aging (Girsang et al., 2019a), CA has the capacity to bind with dermal matrix including matrix metalloproteinase-1 (MMP-1), Neutral Endopeptidase (NEP) and Polyphenol Oxidase-3 (PPO-3) (Girsang et al., 2019a). CA had the highest affinity toward MMP-1, NEP, PPO-3 compared to the others compound of snake fruit peel extract, namely caffeic acid, ferulic acid, protocathecuic acid, and rutin (Girsang et al., 2019a).

CONCLUSION

CA has preventive and curative therapeutic effects on UV-induced fibroblast cells. CA at concentrations 6.25 µg/mL to 25 µg/mL significantly ameliorate cell viability and reduce inhibition either after or before UV exposure. At concentration 6.25 µg/mL, CA results in highest viability cell in both therapies.

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