Research in Pharmaceutical Sciences, December 2024; 19(6): 746-753
Received: 23-08-2022
Peer Reviewed: 06-01-2023
Revised: 03-04-2024
Accepted: 13-11-2024
Published: 15-12-2024

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

Antiaging properties of chlorogenic acid through protein and gene biomarkers in human skin fibroblast cells as photoaging model

Ermi Girsang[1,*], Chrismis N. Ginting [1], I Nyoman Ehrich Lister[1], Wahyu Widowati[2], Afif Yati[3], Hanna Sari Widya Kusuma[3], and Rizal Azis[3,4]

[1]*Faculty of Medicine, Universitas Prima Indonesia, Jl. Sampul No. 4, Medan 20118, North Sumatera, Indonesia.*
[2]*Faculty of Medicine, Maranatha Christian University, Jl. Surya Sumantri No. 65, Bandung 40164, West Java, Indonesia.*
[3]*Biomolecular and Biomedical Research Center, Aretha Medika Utama, Babakan Jeruk II No. 9, Bandung 40163, West Java, Indonesia.*
[4]*Biomedical Engineering, Department of Electrical Engineering, Faculty of Engineering, Universitas Indonesia, Depok, West Java, Indonesia.*

Abstract

Background and purpose: Chlorogenic acid (CA) is a natural chemical that promises antiaging activity against photoaging skin damage. This research examined CA activities in mitigating skin photoaging.
Experimental approach: UV-exposed human skin fibroblast cells were subjected to CA at 6.25, 12.5, and 25 µg/mL. The protein levels of cell secretion, such as cyclooxygenase (COX)-2, nitric oxide (NO), and interleukin (IL)-6 were measured using ELISA and colorimetry methods. Meanwhile, the mRNA expressions of glutathione peroxidase (GPX)-1, tissue inhibitor metalloproteinase (TIMP)-1, matrix metalloproteinase (MMP)-1, caspase (CASP)-3, CASP-8, and fibroblast growth factor (FGF)-2 were quantified using the qRT-PCR method.
Findings/Results: CA treatment reduced inflammatory and aging biomarkers. CA at 6.25 µg/mL lowered NO, COX-2, and IL-6 levels to 89.44 µmol/L, 8.10 ng/mL, and 62.75 pg/mL, respectively. CA at 25 µg/mL resulted in the most significant down-regulation of MMP-1, CASP-3, and CASP-8 genes' expression (3.27, 1.25, and 3.59, respectively). Furthermore, treatment with CA at 25 µg/mL demonstrated the most notable activity in up-regulating antioxidant markers, specifically GPX-1, and extracellular matrix (ECM) integrity markers, including TIMP-1 and FGF-2 genes' expression.
Conclusion and implications: CA imposes its anti-aging activity by decreasing inflammatory and aging biomarkers, and increasing cellular antioxidant and ECM integrity.

Keywords: Antiaging; Chlorogenic acid; Fibroblast; qRT-PCR; Ultraviolet.

INTRODUCTION

Ultraviolet (UV) rays are the major factor in promoting dermal aging by releasing cellular reactive oxygen species (ROS), which generate lipid oxidation, DNA impairment, and protein deformation (1,2) subsequently leading to apoptosis (3). ROS molecule, including nitric oxide (NO), promotes the release of cytokines such as interleukins (IL-6, IL-12, and IL-1β), cyclooxygenase II (COX-2), and tumor necrosis factor-α (TNF-α); activates the nuclear factor-kappa B (NF-kB) cascade; and provokes T cells infiltration to cause inflammation (4). These proteins remarkably contribute to the pathogenesis of skin photoaging. In the study by Surowiak *et al*., COX-2 expression was much higher in keratinocytes and fibroblasts from photoaged skin than in skin samples from younger people or people who were naturally getting older (5).

*Corresponding author: E. Girsang
Tel: +62-82166119002
Email: ermigirsang@unprimdn.ac.id

Access this article online

Website: http://rps.mui.ac.ir

DOI: 10.4103/RPS.RPS_177_22

Besides, IL-6 has recently been identified as a potential target in inflammatory diseases contributing to aging, earning it the moniker "gerontologist's cytokine" (6,7). Furthermore, NO interacts with the superoxide anion (O_2^-) to generate the strong oxidant peroxynitrite ($ONOO^-$), which then interacts with DNA, lipids, and proteins by means of direct oxidation reactions or through indirect mechanisms facilitated by radicals. $ONOO^-$ is associated with various organelle pathologies, such as oxidation, cytotoxicity induction, and apoptosis (8). The ROS-stimulated apoptosis is indicated by the increases in caspase 3 (CASP-3), CASP-8, CASP-6, CASP-9, and CASP-7 (3).

ROS obliterates the antioxidant enzymes, including glutathione peroxidase (GPX); stimulates mitogen-activated protein kinase (MAPK)/activator protein-1 (AP-1) cascade activity, which then activates matrix metalloproteinases (MMPs), and damages tissue integrity (9). An increase in MMPs results in the activation of AP-1. The AP-1 stimulates MMP gene expression while restraining transforming growth factor β (TGF-β) signaling. A continuous MMP activity leads to collagen degradation; hence, it causes lower collagen I levels in photoaging skin (10). As individuals age, MMPs like stromelysin-1 (MMP-3), collagenase-1 (MMP-1), and gelatinase (MMP-2) reduce collagen and elastin fibers within the dermis. Tissue inhibitors of metalloproteinase-1 (TIMP-1) naturally counteract MMP to slow the aging of fibroblasts (11).

Some plant metabolites have anti-aging activity by protecting the skin from oxidation, inflammation, and tissue deformation (10,12). The anti-aging activity is manifested by reducing wrinkles, pigmentation, and skin lines. This repair is caused by promoting dermal growth factor activity, such as fibroblast growth factor 2 (FGF-2). FGF family proteins can increase fibroblast proliferation and activation in skin aging control by promoting the production of type I collagen. FGFs promote angiogenesis and are crucial in cell repair (13). Photoaging therapy may involve inhibiting the expression or activity of an antioxidant enzyme that contributes to the aging process (5). Chlorogenic acid (CA) is considered one of the most promising classes of natural compounds with the ability to control such enzymes and thus have potential as antioxidants and antiaging agents (14).

A phenolic substance, CA 5-O-caffeoylquinic acid), is present in some vegetables and fruits (12,15). Several studies on CA's antioxidant, antitumor, antiviral, anticancer, and anti-inflammatory effects have been so far carried out (16). A prior study reported that CA has antiaging properties by decreasing ROS levels and increasing COL-3 gene expression in a human fibroblast photoaging model (14). However, there is no comprehensive report about the CA effect in mitigating skin aging through assessment of the biomarkers related to oxidative stress, which is concomitant with inflammation, extracellular matrix, and apoptosis. Therefore, this research aimed to find out more about the impact of CA on the antioxidant marker, GPX-1; inflammatory proteins, NO, COX-2, and IL-6; extracellular matrix (ECM) integrity-related genes, MMP-1, TIMP-1, and FGF-2; and cell death markers, CASP-3 and CASP-8.

MATERIALS AND METHODS

Cell culture

Human skin fibroblast cells (BJ, ATCC® CRL-2522TM) were maintained in Aretha Medika Utama, Bandung, Indonesia. It was grown in a medium containing 1% antibiotic/antimycotic (ABAM, L0010-100; Biowest, France), 10% fetal bovine serum (FBS, S1810-500; Biowest, France), 1% amphotericin B (L0009-050; Biowest, France), 0.1% gentamicin (15750060; Gibco, United States), L-glutamine solution (G8540; Sigma-Aldrich, United States), and minimum essential medium (MEM, L0416-500; Biowest, France). The culture was maintained in an incubator set to 37 °C with 5% CO_2. The 80% confluent cells were harvested with 0.25% trypsin-EDTA (25200072; Gibco, United States) for the treatment (14,17).

UV irradiation and treatment

The harvested cells were cultured in six-well plates (n = 10^6 cells) and then incubated for

24 h at 37 ℃ with 5% CO_2 until reaching 80% confluency. The cells were exposed to UVB for 75 mins (300 J/cm^2) once the fresh medium was added. The CA (BP0345; Chengdu Biopurify Phytochemical, China) was added in UV-exposed BJ cells at final concentrations of 6.25, 12.5, and 25 µg/mL The cells were grown for 4 days at 37 ℃ with 5% CO_2, then harvested (13).

Total protein measurement

The bovine serum albumin (BSA) stock was defined as a standard solution. Briefly, 2 mg of BSA (Sigma, A9576, Lot SLB2412, United States) was dissolved in 1000 L ddH_2O to make the stock. A 96-well plate was used for the preparation. A volume of 200 µL Quick Start Dye Reagen 1X (Biorad, 5000205, United States) was introduced to 20 µL of standard solutions and samples in each well. Subsequently, the plate underwent an incubation period of 5 min, followed by the absorbance measurement (595 nm) using a microplate reader (14).

Inflammation protein marker measurement using ELISA and colorimetric assays

Inflammation-related proteins are secreted by the cells into the medium, or "conditioned medium." Therefore, the conditioned medium was measured for the levels of COX-2, IL-6, and NO according to the human IL-6 ELISA kit (E-EL-H 6156, Elabscience, China), human COX-2 ELISA kit (E-EL-H1846, Elabscience, China), and NO colorimetric assay (E-BC-K035-M, Elabscience, China) at 450 nm, 450 nm, and 550 nm, respectively. The absorbance readings were obtained by employing a microplate reader (Multiskan Go, Thermo Scientific) (14,18).

Gene expression analysis

Total cellular RNA extraction was performed under the manufacturer's protocol. AurumTM Total RNA Minikit (732-6820, Bio-Rad) and iScript Reverse Transcription Supermix for quantitative reverse transcriptase polymerase chain reaction (qRT-PCR; 170-8841, Bio-Rad) were used for cDNA synthesis and analyzed using qRT-PCR (G8830A, AriaMx Real-time PCR System). β-actin gene was used to normalize the MMP-1, TIMP-1, CASP-3, CASP-8, FGF-2, and GPX-1 gene expression, and analyzed using AriaMx RT-PCR system with SsoFast Evagreen Supermix (172-5200, Bio-Rad). The reaction mix was based on the previous studies (14,18) with modification on annealing temperature based on optimization of the primer. The primers and RNA properties are shown in Tables S1 and S2.

Statistical analysis

The entire set of data was displayed as mean ± SD. Normally distributed data were processed employing SPSS software (version 20.0). The statistical analysis involved the utilization of ANOVA alongside Tukey's HSD post hoc tests. *P*-values ≤ 0.05 were regarded as statistically significant.

RESULTS

CA effect on protein level

Based on Fig. 1, UV induction led to a decrease in total protein, compared to the negative control. CA treatment at all concentrations could elevate total protein, with CA at 12.5 µg/mL as the most effective. Additionally, all CA treatments exhibited comparable results to the negative control. The total protein levels were used as a benchmark to compare NO, COX-2, and IL-6 levels against the total protein content in the photoaging cells model, providing a reference point for evaluating the effects of CA on protein regulation.

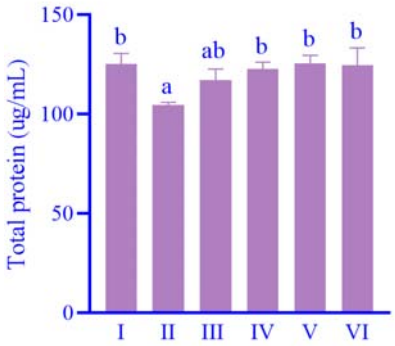

Fig. 1. Effect of CA on protein level in photoaging cells model. Group I: normal cells (negative control); group II: UV-exposed cells (positive control); group III: UV-exposed cells treated with DMSO 1% (vehicle control); and groups IV-VI: UV-exposed cells treated with CA at 6.25; 12.5, and 25 µg/mL, respectively. The data represent mean ± SD. There is no statistically significant difference among the groups with similar alphabetic letters

CA effect on inflammation protein markers

As depicted in Fig. 2A, C, E, UV induction led to alterations of inflammatory markers. UV induction significantly elevated NO, COX-2, and IL-6 levels compared to the negative control, marked an inflammation rise. However, CA was able to regulate corresponding inflammatory markers, notably reducing them. Among three concentrations of CA, 6.25 µg/mL exhibited as the most active in decreasing all target inflammatory markers. Moreover, out of the total protein that could be secreted by the medium, CA has the ability to reduce the levels of all inflammatory markers (Fig. 2B, D, F). The result revealed that CA at 6.25 µg/mL exhibited the most significant reduction in NO, COX-2, and IL-6 levels (728.12 pg/mg protein; 0.07 ng/mg protein; and 510.81 pg/mg protein, respectively).

Antioxidant and aging-related mRNA expressions

The mRNA relative expressions of GPX-1, MMP-1, TIMP-1, FGF-2, CASP-3, and CASP-8 were quantified using the qRT-PCR method. UV exposure significantly up-regulated MMP-1, CASP-3, and CASP-8 and down-regulated GPX-1, TIMP-1, and FGF-2 mRNA expressions compared to the negative control (Fig. 3A-F). In contrast, CA treatment significantly down-regulated MMP-1, CASP-3, and CASP-8, as well as upregulated GPX-1, TIMP-1, and FGF-2 mRNA expressions compared to the positive control. CA at 25 µg/mL was the most effective to down-regulate relative gene expression of MMP-1, CASP-3, and CASP-8 (3.27, 1.25, and 3.59, respectively), and was the most active concentration to upregulate the relative gene expression of GPX-1, TIMP-1, and FGF-2 (0.46, 0.93, and 0.12, respectively).

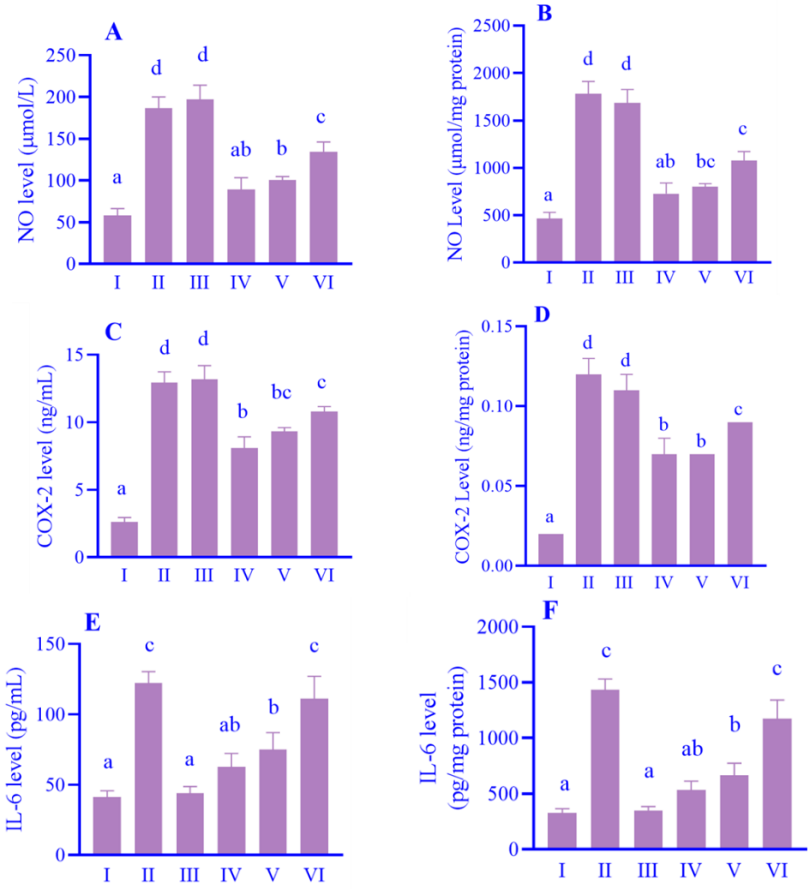

Fig. 2. Effect of chlorogenic acid on NO, COX-2, and IL-6 levels in photoaging cells model. Group I: normal cells (negative control); group II: UV-exposed cells (positive control); group III: UV-exposed cells treated with DMSO 1% (vehicle control); and groups IV-VI: UV-exposed cells treated with CA at 6.25; 12.5, and 25 µg/mL, respectively. The data represent mean ± SD. No significant statistical difference was found among the groups with similar alphabetic letters. NO, Nitric oxide; COX, cyclooxygenase; IL, Interleukin.

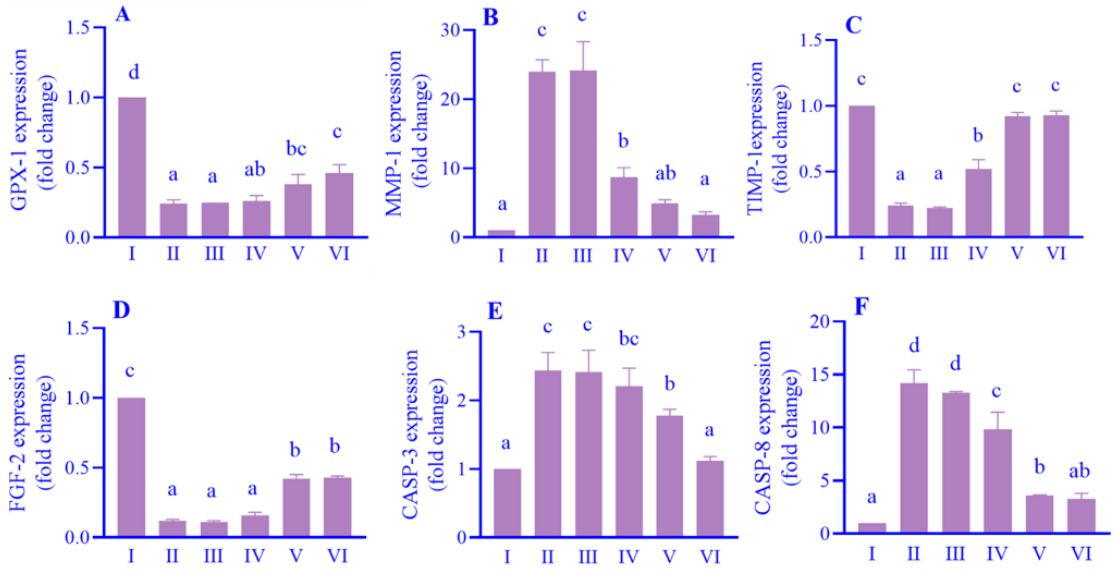

Fig. 3. The effect of chlorogenic acid on relative mRNA expression of GPX-1, MMP-1, TIMP-1, FGF-2, CASP-3, and CASP-8 in photoaging cells model. Group I: normal cells (negative control); group II: UV-exposed cells (positive control); group III: UV-exposed cells treated with DMSO 1% (vehicle control); and groups IV-VI: UV-exposed cells treated with CA at 6.25; 12.5, and 25 µg/mL, respectively. The data represent mean ± SD. No significant statistical difference was found among the groups with similar alphabetic letters. GPX, Glutathione peroxidase; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of matrix; FGF, fibroblast growth factor; CASP, caspase.

DISCUSSION

UV light is one of the causative aspects of facial skin aging (1). Exposure to UV light generates excessive ROS production, activates NF-kB, and activates other aging cascades, such as MMPs and inflammation (19). The current study reported that the levels of NO, COX-2, and IL-6 in the UV-exposed skin fibroblast cells increased indicating oxidative stress and inflammation.

CA is a phenolic component found in coffee, tea, and various fruits and vegetables (12,15). CA is a hydrocinnamic family member and one of the water-soluble phenolic compounds acknowledged as an antioxidant to fight free radicals (20). CA is reported to lower cytokines, including pro-inflammatory ILs, and modulate NF-kB and COX-2 (21). As an indication of mitigation, this study reported that CA up-regulates the antioxidant gene, including GPX-1, and reduces the inflammatory proteins, NO, COX-2, and IL-6 in the UV-exposed skin fibroblast cells. As reported by our previous findings, CA reduced ROS levels in UV-activated human fibroblast skin cells (14) and Pb-induced skin fibroblast cells (17). Additionally, CA isomers significantly lessened DNA damage from the free radical OH*, which increased antioxidant activity (22). CA suppresses IL-6 mediated signaling, which triggers the initiation of the Janus kinase/signal transducer and activator of transcription (JAK/STAT) and NF-kB signaling pathways in the inflammatory response, consequently reducing the levels of the pivotal cytokine involved in these signaling pathways (23 of). CA protects cells from UV-induced inflammation by lowering IL-1 and TNF-α (14). The previous study reported that CA up-regulated the expression of the collagen-forming gene COL3A in UV-induced BJ cells and inhibited cell death as an indication of aging mitigation (14). This study reported the decrease of MMP-1 and the increase of TIMP-1 and FGF-2 mRNA expressions in a photoaging cell model as a CA treatment. The inhibitions of inflammation and ROS generation result in low MMP expression, allowing collagen regeneration in the skin (24). MMP inhibition stimulates TIMP activity to reduce skin photodamage caused by UVB penetration (25). The protection of FGF-2 from degradation can reveal anti-aging activity by significantly improving the elasticity, density, and texture of the skin and reducing wrinkles (13).

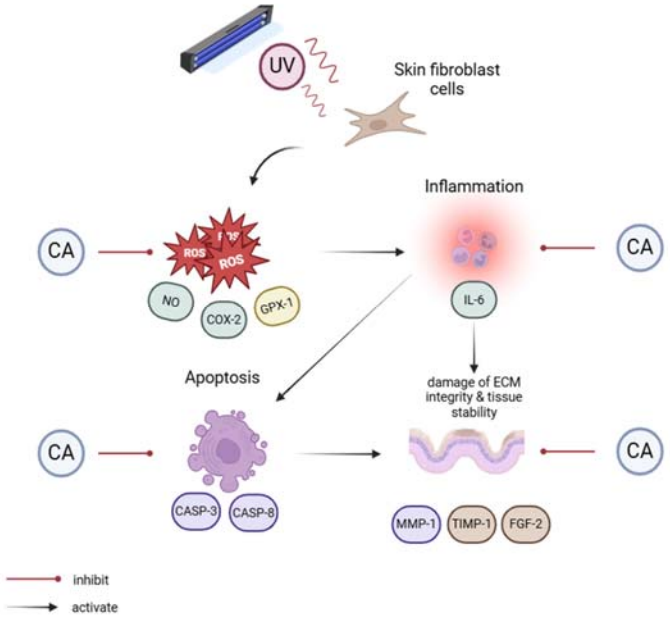

Fig. 4. The proposed anti-aging mechanism in CA treatment. UV exposure generates ROS-excessive production in skin fibroblast cells, leading to oxidative stress and inflammation. Subsequently, the inflammation leads to apoptosis, tissue instability, and ECM degradation. CA treatment reduces NO, COX-2, and IL-6 levels; and enhances GPX-1 production to inhibit oxidative stress and inflammation. This inhibition also keeps skin fibroblast integrity and stability by lowering CASP-3, CASP-8, and MMP-1 and improving TIMP-1 and FGF-2. CA, chlorogenic acid; ROS, reactive oxygen species; ECM, extracellular matrix; NO, nitric oxide; COX, cyclooxygenase; IL, Interleukin; GPX, glutathione peroxidase; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of matrix; FGF, fibroblast growth factor; CASP, caspase.

This study showed that CA reduced cell apoptosis by decreasing the mRNA expressions of the CASP-3 and CASP-8 genes. Several studies have found that CA suppression affects apoptosis. The research by Chen *et al.* showed a decrease in CASP-3 and CASP-9 in the intestines of CA-fed pigs (26). Ali *et al.* found that CA lowered CASP-3 and CASP-9 to stop hepatocyte apoptosis in a rat model of hepatotoxicity (27). The anti-apoptotic effect of CA treatment could be associated with increasing GPX-1, which belongs to the nuclear factor erythroid 2-related factor-2 family. As revealed by Kim *et al.* the increase of NRF-2 contributed to the anti-apoptotic effect of phycocyanin from *Spirulina* to protect skin cells (28).

According to the current study results, CA has potential as an antiaging agent. Current studies have shown that CA has antioxidant and antiapoptotic properties (12), collagenase inhibition activity (29), and collagen regeneration (14,17). The mechanism of the CA antiaging activity against aging fibroblast cells can be seen in Fig. 4.

CONCLUSION

The research revealed that CA has potential as an anti-aging agent in UV-exposed human skin fibroblast cells. CA diminished the levels of pro-inflammatory proteins NO, COX-2, and IL-6; down-regulated dermal degeneration genes MMP-1, CASP-3, and CASP-3; additionally, CA up-regulated antioxidant genes GPX-1 and skin integrity genes TIMP-1 and FGF-2.

Acknowledgments

This study received financial support from the Indonesian Ministry of Education, Culture, Research, and Technology's Penelitian Dasar Unggulan Perguruan Tinggi (PDUPT) 2022 (Grant No. 008/LL1/LT/K/2022). The authors appreciate Aretha Medika Utama, Indonesia, for the technical and facility support.

Conflict of interest statement

The authors stated that there were no conflict of interest in this study.

Authors' contributions

E. Girsang, C.N. Ginting, I.N.E. Lister, and W. Widowati conceived the research design. A. Yati and H.S.W. Kusuma conducted the research. W. Widowati and R. Azis collected the data. C.N. Ginting, I.N.E. Lister, W. Widowati, and A. Yati drafted the manuscript. E. Girsang, W. Widowati, and R. Azis finalize the manuscript. The final version of the manuscript was reviewed and approved by all authors.

REFERENCES

1. Pattananandecha T, Apichai S, Julsrigival J, Ungsurungsie M, Samuhasaneetoo S, Chulasiri P, *et al*. Antioxidant activity and anti-photoaging effects on UVA-irradiated human fibroblasts of rosmarinic acid enriched extract prepared from *Thunbergia laurifolia* leaves. Plants. 2021;10(8):1-12. DOI: 10.3390/plants10081648.
2. Xuan SH, Lee NH, Park SN. Atractyligenin, a terpenoid isolated from coffee silverskin, inhibits cutaneous photoaging. J Photochem Photobiol B. 2019;194:166-173. DOI: 10.1016/j.jphotobiol.2019.04.002.
3. Redza-Dutordoir M, Averill-Bates DA. Activation of apoptosis signalling pathways by reactive oxygen species. Biochim Biophys Acta. 2016;1863(12):2977-2992. DOI: 10.1016/j.bbamcr.2016.09.012.
4. Man MQ, Wakefield JS, MauroTM, Elias PM. Regulatory role of nitric oxide in cutaneous inflammation. Inflammation. 2022;45:949-964. DOI: 10.1007/s10753-021-01615-8.
5. Surowiak P, Gansukh T, Donizy P, Halon A, Rybak Z. Increase in cyclooxygenase-2 (COX-2) expression in keratinocytes and dermal fibroblasts in photoaged skin. J Cosmet Dermatol. 2014;13(3):195-201. DOI: 10.1111/jocd.12103.
6. Greene MA, Loeser RF. Aging-related inflammation in osteoarthritis. Osteoarthritis Cartilage. 2015;23(11):1966-1971. DOI: 10.1016/j.joca.2015.01.008.
7. Rea IM, Gibson DS, McGilligan V, McNerlan SE, Alexander HD, Ross OA. Age and age-related diseases: role of inflammation triggers and cytokines. Front Immunol. 2018;9:586,1-28. DOI: 10.3389/fimmu.2018.00586.
8. Pourbagher-Shahri AM, Farkhondeh T, Talebi M, Kopustinskiene DM, Samarghandian S, Bernatoniene J. An overview of NO signaling pathways in aging. Molecules. 2021;26(15):4533,1-46. DOI: 10.3390/molecules26154533.
9. Choi SI, Lee JH, Kim JM, Jung TD, Cho BY, Choi SH, *et al*. *Ulmus macrocarpa hance* extracts attenuated H_2O_2 and UVB-induced skin photo-aging by activating antioxidant enzymes and inhibiting MAPK pathways. Int J Mol Sci. 2017;18(6):1-14. DOI: 10.3390/ijms18061200.
10. Warsito MF, and Kusumawati IK. The impact of herbal products and the prevention, regeneration, and delay of skin aging. Adv Exp Med Biol. 2019;1178 (9):155-174. DOI: 10.1007/978-3-030-25650-0_9.
11. Shin JW, Kwon SH, Choi JY, Na JI, Huh CH, Choi HR, *et al*. Molecular mechanisms of dermal aging and antiaging approaches. Int J Mol Sci. 2019;20(9):2126,1-16. DOI: 10.3390/ijms20092126.
12. Kageyama H, Waditee-Sirisattha R. Antioxidative, anti-inflammatory, and anti-aging properties of Mycosporine-like amino acids: molecular and cellular mechanisms in the protection of skin-aging. Mar Drugs. 2019;17(4):222:1-18. DOI: 10.3390/md17040222.
13. de Araújo R, Lobo M, Trindade K, Silva DF, Pereira N. Fibroblast growth factors: a controlling mechanism of skin aging. Skin Pharmacol Physiol. 2019;32(5):275-282. DOI: 10.1159/000501145.
14. Girsang E, Ginting CN, Lister INE, Gunawan KY, Widowati W. Anti-inflammatory and antiaging properties of chlorogenic acid on UV-induced fibroblast cell. Peer J. 2021;9:1-15. DOI: 10.7717/peerj.11419.
15. Naveed M, Hejazi V, Abbas M, Kamboh AA, Khan GJ, Shumzaid M, *et al*. Chlorogenic acid (CGA): a pharmacological review and call for further research. Biomed Pharmacother. 2018;97:67-74. DOI: 10.1016/j.biopha.2017.10.064.
16. Changizi Z, Moslehi A, Rohani AH, Eidi A. Chlorogenic acid inhibits growth of 4T1 breast cancer cells through involvement in Bax/Bcl2 pathway. J Cancer Res Ther. 2020;16(6):1435-1442. DOI: 10.4103/jcrt.JCRT_245_19.
17. Girsang E, Lister INE, Ginting CN, Nasution SL, Suhartina S, Munshy UZ, *et al*. Antioxidant and anti-inflammatory activity of chlorogenic acid on lead-induced fibroblast cells. J Phys. 2019;1374:1-9. DOI: 10.1088/1742-6596/1374/1/012006.
18. Pujimulyani D, Suryani CL, Setyowati A, Handayani RAS, Arumwardana S, Widowati W, *et al.* Cosmeceutical potentials of *Curcuma mangga Val.* extract in human BJ fibroblasts against MMP-1, MMP3, and MMP-13. Heliyon. 2020;6(9):1-6. DOI: 10.1016/j.heliyon.2020.e04921.
19. Tang X, Yang T, Yu D, Xiong H, Zhang S. Current insights and future perspectives of ultraviolet radiation (UV) exposure: friends and foes to the skin and beyond the skin. Environ Int. 2024;185(2024):1-15. DOI: 10.1016/j.envint.2024.108535.
20. Santana-Gálvez J, Cisneros-Zevallos L, Jacobo-Velázquez DA. Chlorogenic acid: recent advances on its dual role as a food additive and a nutraceutical against metabolic syndrome. Molecules. 2017;22(3):358,1-21. DOI: 10.3390/molecules22030358.
21. Liang N, Kitts DD. Role of chlorogenic acids in controlling oxidative and inflammatory stress conditions. Nutrients. 2016;8(1):16;1-20. DOI: 10.3390/nu8010016.

22. Tomac I, Šeruga M, Labuda J. Evaluation of antioxidant activity of chlorogenic acids and coffee extracts by an electrochemical DNA-based biosensor. Food Chem. 2020;325:1-25. DOI: 10.1016/j.foodchem.2020.126787.
23. Lou L, Zhou J, Liu Y, Wei Y, Zhao J, Deng J, *et al*. Chlorogenic acid induces apoptosis to inhibit inflammatory proliferation of IL-6 induced fibroblast-like synoviocytes through modulating the activation of JAK/STAT and NF-kB signaling pathways. Exp Ther Med. 2016;11(5):2054-2060. DOI: 10.3892/etm.2016.3136.
24. Pham TLB, Thi TT, Nguyen HTT, Lao TD, Binh NT, Nguyen QD. Anti-aging effects of a serum based on coconut oil combined with deer antler stem cell extract on a mouse model of skin aging. Cells. 2022;11(4):597,1-15. DOI: 10.3390/cells11040597.
25. Hwang I, Choi KA, Kim M, Hong S. Neural stem cells and the secreted proteins TIMPs ameliorate UVB-induced skin photodamage. Biochem Biophys Res Commun. 2019;518(2):388-395. DOI: 10.1016/j.bbrc.2019.08.068.
26. Chen J, Xie H, Chen D, Yu B, Mao X, Zheng P, *et al*. Chlorogenic acid improves intestinal development *via* suppressing mucosa inflammation and cell apoptosis in weaned pigs. ACS Omega. 2018;3(2):2211-2219. DOI: 10.1021/acsomega.7b01971.
27. Ali N, Rashid S, Nafees S, Hasan SK, Shahid A, Majed F, *et al*. Protective effect of chlorogenic acid against methotrexate induced oxidative stress, inflammation, and apoptosis in rat live: an experimental approach. Chem-Biol Interact. 2017;272:80-91. DOI: 10.1016/j.cbi.2017.05.002.
28. Kim KM, Lee JY, Im AR, Chae S. Phycocyanin protects against UVB-induced apoptosis through the PKC α/βII-Nrf-2/HO-1 dependent pathway in human primary skin cells. Molecules. 2018; 23(2):478:1-12. DOI: 10.3390/molecules23020478.
29. Girsang E, Lister INE, Ginting CV, Sholihah IA, Raif MA, Kunardi S, *et al*. Antioxidant and antiaging activity of rutin and caffeic acid. Pharmaciana. 2020;10(2):147-156. DOI: 10.12928/pharmaciana.v10i2.13010.

SUPPLEMENTARY MATERIALS

Table S1. Primer sequences

Genes	Primer sequence (5' to 3')	Size (bp)	Annealing (℃)	Cycle	NCBI references sequence
β-actin	Forward: 5'-TCTGGCACCACACCTTCTACAATG-3' Reverse: 5'-AGCACAGCCTGGATAGCAACG-3'	230	60	40	NM_001101.5
MMP1	Forward: 5'-AGTCCAAGAGAATGGCCGAG-3' Reverse: 5'-CTGAAGGTGATGAAGCAG-3'	230	60	40	NM_001191642.1
TIMP1	Forward: 5'-TTTCAGAGCCTTGGAGGAGCTGGTC-3' Reverse: 5'-AGTCAACCAGACCACCTTATACCA-3'	230	60	40	NM_003254.3
Casp3	Forward: 5'-CAGTGTTCTCCATGGATACCTTTATT-3' Reverse: 5'-CTGGTTTTCGGTGGGTGT-3'	230	60	40	NM_001354783.2
Casp8	Forward: 5'-GGTCACTTGAACCTTGGGAA-3' Reverse: 5'-AGGCCAGATCTTCACTGTCC-3'	230	60	40	NM_001400660.1
FGF-2	Forward: 5'-GGCTTCTTCCTGCGCATCCA-3' Reverse: 5'-GCTCTTAGCAGACATTGGAAGA-3'	230	58	40	NM_002006.6
GPX-1	Forward: 5'-CCAAGCTCATCACCTGGTCT-3' Reverse: 5'-TCGATGTCAATGGTCTGGAA-3'	230	58	40	NM_001329455.2

MMP, matrix metalloproteinase; TIMP, tissue inhibitor of matrix; FGF, fibroblast growth factor; Casp, caspase; GPX, glutathione peroxidase.

Table S2. RNA properties used for cDNA synthesis

Treatment	RNA concentration (ng/mL)	RNA purity (λ260/λ280 nm)
Negative control	627.25	2.1690
Positive control	80.85	2.2518
DMSO control	102.1	2.1701
Chlorogenic acid (6.25 µg/mL)	252.20	2.2660
Chlorogenic acid (12.5 µg/mL)	179.55	2.3541
Chlorogenic acid (25 µg/mL)	163.25	2.2754