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Research Article

Photoprotective Effect of *Samia ricini* (Drury, 1773) Silkworm Cocoon Extract on Viability and Collagen Production in Human Dermal Fibroblast (HDF) Cells Induced by UVB Irradiation

Cintya Angelina[1], Slamet Widiyanto[2]*, Ardaning Nuriliani[3], Sukirno Sukirno[4]
1)Faculty of Biology, Universitas Gadjah Mada. Jl. Teknika Selatan, Sekip Utara, Yogyakarta 55281, Indonesia
2)Laboratory of Animal Physiology, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia 55281
3)Laboratory of Animal Structure and Development, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia 55281
4)Laboratory of Entomology, Faculty of Biology, Universitas Gadjah Mada. Jl. Teknika Selatan, Sekip Utara, Yogyakarta 55281, Indonesia
* Corresponding author, email: slametbio@ugm.ac.id

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ABSTRACT

UVB radiation (290–320 nm) induces oxidative stress, reducing cell viability and degrading collagen in human dermal fibroblasts (HDF). *Samia ricini*, a non-mulberry silkworm, produces sericin—a natural protein with antioxidant, UV-protective, and anti-aging properties. However, its photoprotective potential remains underexplored, particularly in Indonesia. This study investigates the protective effects of *Samia ricini* cocoon extract (EKUS) against UVB-induced damage in HDF cells, as evaluated by measuring cell viability using the MTT assay and collagen production through the Sirius Red assay. HDF cells seeded at 5×10^3 and 1×10^4 cells/well were irradiated with UVB (280 mJ cm^{-2}) and treated with EKUS at concentrations ranging from 15.625 to 1000 µg mL^{-1}. EKUS showed no cytotoxicity in normal HDF cells across this concentration range. Pre-treatment with 500 µg mL^{-1} EKUS maintained cell viability above 80 % post-UVB exposure. At 1000 µg mL^{-1}, EKUS significantly enhanced cell viability to 123.86 ± 16.77 % and 128.39 ± 13.22 % at 5×10^3 and 1×10^4 cells/well, respectively. In collagen assays, EKUS at 900 µg mL^{-1} increased collagen production to 146.30 ± 27.20 % (5×10^3 cells/well) and 189.04 ± 9.66 % (1×10^4 cells/well), compared to UVB-treated controls. These findings indicate that EKUS exhibits significant photoprotective effects by preserving cell viability and enhancing collagen synthesis in UVB-exposed HDF cells. Additional studies are suggested to confirm its potential in more intricate models.

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INTRODUCTION

The skin, a crucial organ, plays a major protective role against environmental stressors such as pathogens, infections, chemicals, and ultraviolet (UV) rays. UV radiation consists of UVA (315–400 nm), UVB (290–320 nm), and UVC (100–280 nm). UVB radiation has the potential to damage the DNA and protein structure of skin cells, particularly in the epidermis (Salminen et al. 2022). UVB rays predominantly affect the skin, leading to increased production of matrix metalloproteinases (MMPs) and reduced collagen synthesis, resulting in photoaging (Jung et al. 2014). Photoaging is a condition of damage resulting from chronic exposure to UV light, which contributes to intrinsic skin aging (Pandel et al. 2013). UVB can elevate the production of reactive oxygen species (ROS) in skin cells, which can manifest possible detrimental effects on skin health (Amaro-ortiz et al. 2014). Thus, the skin's defence against increased ROS caused by UVB radiation must be maintained by an antioxidant system, which functions to fight ROS and maintain redox balance in the body (Kumar et al. 2018). UVB radiation is recognized for causing direct DNA damage and the production of ROS, which can lead to cellular senescence, apoptosis, and inflammation. In the case of Human Dermal Fibroblast (HDF) cells, UVB-induced ROS production contributes to the degradation of collagen, a major extracellular matrix protein responsible for skin's structural integrity and elasticity. Prolonged UV exposure disrupts collagen synthesis, leads to the breakdown of collagen fibres, and impairs the normal function of fibroblasts. These processes contribute to the visible signs of skin ageing, such as wrinkling and loss of skin firmness (Cavinato & Jansen-Dürr 2017). The antioxidant system, therefore, plays an essential role in protecting fibroblasts from oxidative damage caused by UV exposure and maintaining collagen homeostasis, which is vital for skin health and resilience.

Sericin, present in numerous silkworm cocoons, exhibits antioxidant properties (Liu et al. 2022) and provides protection against UV rays (Sukirno et al. 2022). Extracts of sericin from mulberry (*Antheraea assamensis*) and non-mulberry (*Philosamia ricin*) silkworm cocoons, at concentrations of 10 and 100 µg mL^{-1}, when administered UVB (120 mJ cm^{-2}) irradiation to HaCaT cells, exhibit UV-protective properties. This is achieved by enhancing cell viability by regulating IL-6 (Interleukin 6) and IL-8 (Interleukin 8), increasing p53, and reducing dysregulation of BCL-2/BAX gene expression (Kumar et al. 2018). Sericin also increases collagen production in HDF cells through MMP-2 regulation without having a cytotoxic effect, as well as increasing cell viability in UVB-irradiated HaCaT cells (Kanpipit et al. 2022). Sericin's antioxidant activity is mainly attributed to its ability to eliminate reactive oxygen species (ROS). Additionally, the presence of arginine and alanine residues enables sericin to interact with tyrosinase, an enzyme involved in melanogenesis, thereby exerting anti-tyrosinase activity. This dual function of antioxidant and enzyme-inhibitory action positions sericin as a promising bioactive compound for skin protection and anti-photoageing applications (Aad et al. 2024). Based on this, sericin derived from silkworm cocoons holds promise as a potential agent for protecting skin from ageing caused by exposure to UVB rays.

However, the protective activity of non-mulberry *Samia ricini* silkworm cocoon sericin extract (EKUS) against skin damage caused by UVB rays has not yet been widely explored in Indonesia. Thus, the aim of this study is to assess the ability of EKUS to protect human dermal fibroblast (HDF) cells from specific doses of UVB radiation. Sericin extract obtained from the *Samia ricini* cocoons has potential as a therapeutic agent aimed at protecting skin cells and treating skin problems caused by UVB exposure. This potential can be applied to the development of more innovative and sustainable skincare products for photoageing prevention.

MATERIALS AND METHODS

Materials

Sirius Red staining, MTT (Thiazolyl blue tetrazolium bromide), and complete DMEM Low Glucose medium, which contains 10 % FBS, 0.5 % fungizone (Gibco), and 2 % penicillin-streptomycin (Gibco), dimethyl sulfoxide (DMSO) (Merck), 0.25 % trypsin-EDTA, aquades, sodium bicarbonate (HEPES), 10 % solution of SDS (sodium dodecyl sulfate) in 0.01 N HCl, ethanol (Merck GmbH), Dulbecco's phosphate buffered saline (PBS), Bouin's solution.

Methods

Cell Culture

Human dermal fibroblast (HDF) cells were obtained from the Department of Dermatology and Venereology, Faculty of Medicine, Public Health, and Nursing at Universitas Gadjah Mada (UGM). The cells were maintained in DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10 % heat-inactivated fetal bovine serum (FBS), along with 100 U mL^{-1} penicillin and 100 µg mL^{-1} streptomycin (Invitrogen, Carlsbad, CA, USA). The cells were incubated at 37 °C in a humidified environment containing 5 % CO_2. Subculturing was performed using trypsinisation to establish a new culture. This protocol received approval from the Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada (KE/FK/1170/EC/2023).

Extraction of *Samia ricini* Cocoon

Cocoons of *Samia ricini* were obtained from the Jantra Mas Sejahtera (JAMTRA) wild silk production facility in Yogyakarta, Indonesia. The extraction method was modified based on the procedure described by Rocha et al. (2017). Cocoon pieces (15 g) were autoclaved in 1000 mL of ultrapure water (aquatridest) at 120 °C for 60 minutes. The resulting solution was vacuum-filtered using a Buchner funnel. The filtrate was aliquoted into multiple glass flasks and subsequently frozen at −86 °C and lyophilised for 48 hours. The crude extract was then ground using a mortar and kept at −20 °C until further use. Based on an average cocoon weight of 0.2–0.3 grams, approximately 50 to 75 cocoons were used in this extraction. The larvae producing these cocoons typically have a body weight range of 2–3 grams during the final instar stage.

For biological assays, serial dilutions of the crude extract (EKUS) were prepared by diluting the stock solution in complete medium, ensuring that the DMSO concentration did not surpass 1 %.

Optimization of UVB Irradiation

Control and treated cells were washed and subsequently covered with phosphate-buffered saline prior to UVB irradiation, and irradiated with UVB light (Broadband/Narrowband Ultraviolet B, Puslit KIM – LIPI) with a wavelength of 290 nm at a distance of 40 cm. After UVB radiation at different dosages (70, 140, 280 mJ cm^{-2}), the cells were rinsed three times with phosphate-buffered saline, then incubated at 37 °C for 24 hours in a humidified atmosphere containing 5 % CO_2. The specific UVB dose is interpreted in terms of exposure time (seconds), which is calculated based on the calibration by Laboratory of Dermatology and Venereology, Faculty of Medicine, Public Health, and Nursing, University of Gadjah Mada.

Cytotoxicity test of cocoon extract on normal HDF cells

HDF cells (5×10^3 and 10^4 cells per well) were plated in a 96-well tissue culture plate and cultured until they reached 80–90 % confluency. EKUS at con-

centrations of 0;15,625; 31,25; 62,5; 125; 250; 500; and 1000 µg mL^{-1} was applied to the cells for 24 hours at 37 °C in a humidified environment with 5 % CO_2. Afterwards, the cells were treated with MTT solution for 4 hours, after which the formazan crystals were dissolved in 10 % SDS. The optical density was measured using a multiplate reader (Bio-Rad) at 570 nm.

Cytoprotective test using the MTT assay

To assess the potential of EKUS in protecting HDF cells from UVB radiation, the cells were pre-treated with 0;15,625; 31,25; 62,5; 125; 250; 500; and 1000 µg mL^{-1} EKUS for 24 hours, followed by exposure to UVB light at dosage 280 mJ cm^{-2} (344 sec), then cultured at 37 °C for 24 hours. Afterward, MTT stock solution was introduced into each well. After incubating the cells for four hours, the medium were removed. The formazan deposits present in each well were solubilised with 10 % SDS, and the absorbance was measured at 570 nm.

Analysis of collagen production with Sirius red assay

The collagen production test followed the method of Szász et al. (2023), with modifications. HDF cells were incubated with EKUS for 48 h, and exposed to UVB (280 mJ cm^{-2}). The culture medium was removed, subsequently, the cells were rinsed with 200 µL of phosphate-buffered saline (PBS) followed by fixation using Bouin's solution at room temperature overnight. Following an additional wash with 200 µL of PBS, cell staining was performed by introducing 50 µL of a 0.1 % Sirius Red (Direct Red 80) solution dissolved in 1 % acetic acid. The plates were allowed to stand at room temperature for 1 hour, after which the wells were rinsed with 0.1 M HCl. Next, each well was treated with a 0.1 M NaOH solution to release the dye attached to the collagens associated with the cells. The optical density (OD) of the solutions was then measured at 550 nm using a Tecan Spark 20M microplate reader.

Statistical analysis

Each measurement was carried out in triplicate, and the results are presented as the mean ± standard error. Statistical analysis was performed using a one-way analysis of variance (ANOVA), subsequently analysed using Duncan's post hoc test to evaluate differences among the means, assuming normality and homogeneity of variances.

RESULTS AND DISCUSSION

Samia ricini (Drury, 1773) Silkworm Cocoon Extraction

The extraction of sericin using autoclaving followed by lyophilisation produced a brownish powder (Figure 1), indicating successful separation of sericin from the *Samia ricini* cocoon. This method is widely regarded as simple and efficient, yielding a clean extract suitable for further applications (Chlapandias et al. 2013). In this study, the extract yield was approximately 4.3 g, equivalent to 28.6 % (w w^{-1}), which is notably higher than the 18 % (w w^{-1}) yield reported by Rocha et al. (2017) using a similar method. This difference may be attributed to variations in the freezing/thawing process prior to lyophilisation, which influences fibroin deposition and sericin separation from the cocoon.

Although effective, autoclaving can degrade sericin due to high heat and pressure. This degradation occurs through peptide bond hydrolysis, breaking the protein into smaller fragments (Gupta et al. 2014). Such changes may reduce its antioxidant activity and overall biofunction, especially with prolonged or excessive heat, which can denature the protein and damage key amino acids critical for its protective role. The extent of degradation is influenced by several parameters, particularly the temperature and duration of the

autoclave cycle. For example, Wang et al. (2021) demonstrated that extraction yields increase with temperature, reporting a maximum yield of 36.5 % ± 0.4 % at 220 °C. However, such high temperatures also increase the risk of protein breakdown. In contrast, extraction at 120 °C, as reported by Gimenes et al. (2014), typically yields between 21 % and 30 %, offering a compromise between yield and protein integrity. Therefore, careful optimisation of autoclaving conditions is essential. In this study, the conditions were controlled to minimise degradation, balancing between maintaining sericin's functional properties and achieving a high yield.

To maintain sericin's bioactivity, it is important to use the lowest effective autoclaving temperature and shortest duration, followed by immediate lyophilisation to reduce heat and moisture exposure. Rocha et al. (2017) showed that this method preserves antioxidant activity. Given sericin's capacity to counteract free radicals and diminish oxidative stress, this study explores the protective effect of *Samia ricini* sericin extract (EKUS) to prevent UVB-triggered damage in HDF.

Figure. 1 *Samia ricini* silkworm cocoon extraction results.

Optimization of UVB Irradiation

An increasing trend in UVB dose appeared to reduce HDF cell viability, although no statistically significant differences were observed ($p > 0.05$) (Figure 2A). HDF cells were subjected to UVB exposure for 86, 172, and 344 seconds, corresponding to energy doses of 70, 140, and 280 mJ cm^{-2}, respectively. At a seeding density of 5×10^3 cells/well, cell viability was recorded as 90.06 % ± 14.42, 85.85 % ± 17.41, and 78.34 % ± 8.85 for each respective dose. Despite the lack of statistical significance, these values indicate a clear dose-dependent downward trend, suggesting potential biological relevance. This trend aligns with findings by Zheng et al. (2019), who reported that UVB-induced reductions in HDF viability depend on both the dose and duration of exposure. UVB radiation is known to exert cytotoxic effects by promoting DNA damage, increasing reactive oxygen species (ROS), impairing collagen homeostasis, and disrupting proteasome activity—mechanisms that contribute to cellular senescence and apoptosis (Cavinato & Jansen-Dürr 2017).

The viability decrease at 280 mJ cm^{-2} did not exceed 50 %, but it still represented a substantial reduction of approximately 24–30 % compared to the control group. This partial yet measurable decline in cell viability was deemed suitable for further analysis, especially for assessing the protective effects of compounds such as EKUS. Using a dose that causes complete cytotoxicity could obscure potential protective responses, while a sub-lethal but biologically significant dose like 280 mJ cm^{-2} provides an optimal condition to evaluate cellular responses to damage and recovery.

The selection of 280 mJ cm^{-2} is further supported by previous studies. Debacq-Chainiaux et al. (2005) described sub-cytotoxic effects at 250 mJ cm^{-2}, which were sufficient to induce stress responses without total loss of viability.

Similarly, Dobrzyńska et al. (2016) reported 200 mJ cm^{-2} as an efficient dose to induce measurable UVB-induced cellular damage in fibroblasts. Based on these findings, 280 mJ cm^{-2} represents a physiologically relevant and biologically effective dose that triggers measurable cell stress and viability reduction while avoiding complete cytotoxicity, making it suitable for evaluating the potential protective effects of EKUS.

Additionally, seeding density was shown to influence UVB response. At a higher cell density of 1×10^4 cells/well, viability initially increased with doses up to 140 mJ cm^{-2} but decreased at 280 mJ cm^{-2} (Figure 2B). These observations suggest that cell-to-cell contact, UV absorption, and the distribution of UV-sensitive components such as chromophores can influence outcomes (Cavinato & Jansen-Dürr 2017). Thus, while statistical significance was not achieved in this initial assay, the biological relevance of the chosen UVB dose is strongly supported by the literature and forms a rational basis for testing the protective efficacy of EKUS in subsequent experiments.

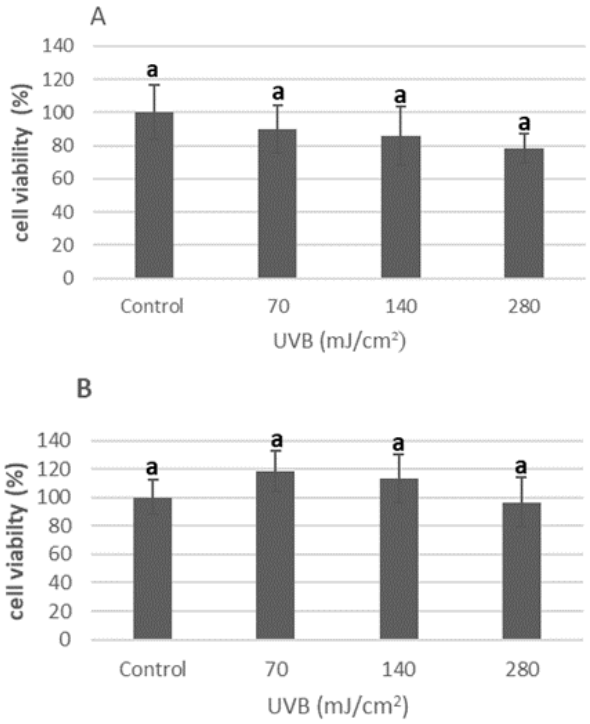

Figure 2. Effect of UVB radiation on HDF cell viability (A) cell density $5x10^3$ cells/well (B) density of 10^4 cells/well evaluated using the MTT assay.

Cytotoxic effect of EKUS on normal HDF cells

EKUS was dissolved in 1 % DMSO because it did not affect drug binding but increased the solubility of the compound (Nguyen et al. 2020). The results of this study indicated that the percentage of live HDF cells in DMSO treatment (Figure 3) at a density of $5x10^3$ and 10^4 cells/well was 84.07 ± 15.97 % and 103.70 ± 12.49 %, respectively. This shows that cells remain viable at a DMSO concentration of 1 %, indicating it is not toxic. This is consistent with the study conducted by Nguyen et al. (2020), which reported that using DMSO at concentration of 0.5-3 % as a drug solvent for the proliferation of skin fibroblast cells does not cause cell death.

EKUS preserved HDF cell viability above 80 % across all tested concentrations (Figure 3). It demonstrated non-toxicity towards HDF cells, with cell viability showing a tendency to rise with increasing EKUS concentration. The data revealed that no meaningful difference was observed ($p\leq0.005$) between EKUS concentrations on cell viability at a density of $5x10^3$ cells/well (Figure 3A.) when contrasted with the control cells. However, HDF cells

treated with EKUS 250, 500 and 1000 µg mL^{-1} increased cell viability by 86.11 ± 12.40 %; 89.93 ± 18.06 % and 117.96 ± 18.78 %, respectively (Figure 3A) High concentrations of EKUS (500-1000 µg mL^{-1}) significantly ($p \leq 0.005$) increased cell viability to <160 % at a cell density of 10^4 cells/well (Figure 3B). This result aligns with previous studies, such as Rocha et al. (2017), which showed that the highest concentration of cocoon extract, when prepared using lyophilisation, exhibited mitochondrial restoration activity in HUVEC cells. The role of sericin in regulating cell growth and viability is concentration-dependent, and in fibroblasts, *S. ricini* sericin has been noted to enhance cell proliferation within 3 days (Sahu et al. 2016). Similarly, sericin extracted from other silk species, such as *Antheraea assamensis*, *Philosamia ricini*, and *Bombyx mori*, has been shown to significantly increase mouse fibroblast proliferation at concentrations up to 400 µg mL^{-1} (Kumar & Mandal 2017). These findings support the conclusion that EKUS promotes cell proliferation without inducing toxicity in HDF cells. However, it is important to consider the potential side effects and long-term toxicity of EKUS, particularly at higher concentrations. Future studies should evaluate these aspects to ensure the safety and efficacy of EKUS for therapeutic use, especially regarding its prolonged exposure or chronic use in clinical applications.

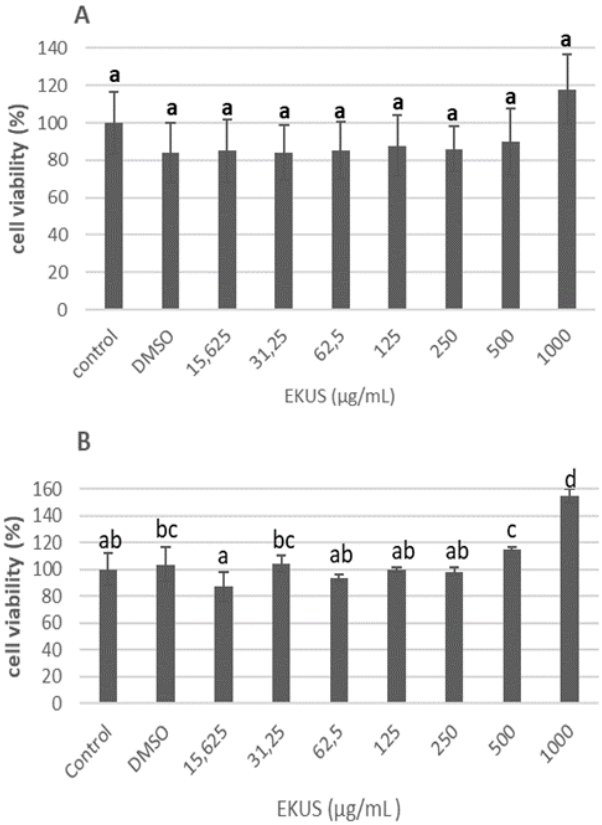

Figure 3. Cytotoxicity effect of EKUS on HDF cells (A) density $5x10^3$ cells/well (B) density 10^4 cells/well.

Protective activity of EKUS on HDF cells against UVB irradiation

The primary objective of this research was to evaluate the cytoprotective activity of EKUS in human dermal fibroblasts (HDF) subjected to UVB radiation at a dose of 280 mJ cm^{-2}. UVB irradiation is known to induce cellular damage through mechanisms like increased ROS production, DNA damage, and the initiation of apoptosis. Sericin, a protein found in silk, has been recognised for its antioxidant properties, which help neutralise free radicals and mitigate UVB-induced damage (Kumar et al. 2018). In this study, EKUS was tested for its ability to protect HDF cells from UVB-induced cell death.

The results indicated a trend towards reduced cell viability with in-

creasing UVB exposure, with a 20–30 % decrease in viability compared to control cells. However, this decline was not found to be statistically significant ($p>0.05$) (Figure 4). This aligns with the work of Sajeeda et al. (2024), who observed that UVB exposure modulates the expression of key apoptotic proteins, including a decrease in the anti-apoptotic protein BCL-2 and an up-regulation of the pro-apoptotic protein BAX in HDF cells. While BAX facilitates cell death, BCL-2 prevents apoptosis by counteracting BAX's activity (Khodapasand et al. 2015). In our study, EKUS treatment, at concentrations below 500 µg mL^{-1}, did not significantly modulate the BCL-2/BAX expression profile, nor did it prevent the DNA damage induced by UVB. This suggests that while EKUS contains antioxidant compounds, they may not be sufficient at these concentrations to fully counteract the dysregulation of these apoptotic pathways.

Interestingly, EKUS at higher concentrations (500–1000 µg mL^{-1}) significantly improved cell viability, maintaining it above 80 % and up to 128.39 % ± 13.22 % at a concentration of 1000 µg mL^{-1}, the highest tested (Figures 4A and 4B). These findings suggest that EKUS can offer significant protection against UVB-induced damage at higher concentrations. This protective effect may be attributed to the cumulative antioxidant activity of sericin, which, at these concentrations, is sufficient to reduce ROS levels and promote cell survival. The antioxidant properties of sericin are mainly due to its ability to neutralize reactive oxygen species (ROS), a function that is amplified by its high levels of polar amino acids like serine and threonine. These amino acids contribute to metal-chelating activity by binding transition metal ions like copper and iron, which are known to catalyse ROS generation via Fenton-type reactions (Aad et al. 2024). These findings are in agreement with previous research by Kumar et al. (2018), who showed that silk sericin extracted from *Philosamia ricini*, a non-mulberry silkworm species, was effective at protecting cells from UV radiation at lower concentrations. However, the discrepancies in protective effects between our study and Kumar et al. (2018) are likely due to differences in extraction methods. The lyophilisation method used in our study may have produced a sericin extract with higher bioactivity compared to the methods used by Kumar et al. (2018), who observed differing effects of sericin based on its extraction method.

Despite these promising findings, the study has several limitations. First, only one cell type, HDF, was utilized in this study. While HDF cells are an important model for studying skin responses, they do not fully capture the complexity of skin's response to UVB radiation, which involves multiple cell types such as keratinocytes. Therefore, the observed protective effects of EKUS may not necessarily extend to other cell types involved in skin damage. Second, this study was conducted entirely in vitro, which limits the translational value of the results. While HDF cell cultures provide a controlled environment for studying cellular mechanisms, they fail to replicate the in vivo complexity of UVB exposure, such as skin's multi-layered structure, the role of immune responses, and the impact of environmental factors. Future research using in vivo models will be essential for a more thorough understanding of the systemic effects and efficacy of EKUS in protecting skin from UVB-induced damage.

While EKUS showed protective effects at higher concentrations, optimizing dosage and treatment time is needed to determine the most effective level for skin protection. Further research should also explore the molecular pathways involved, including antioxidant and anti-inflammatory mechanisms. In conclusion, EKUS effectively protected HDF cells from UVB-induced damage at higher concentrations, significantly improving cell viability. However, limitations such as the use of a single cell type and the absence of in vivo studies highlight the need for further research in more complex models to validate its efficacy and mechanisms.

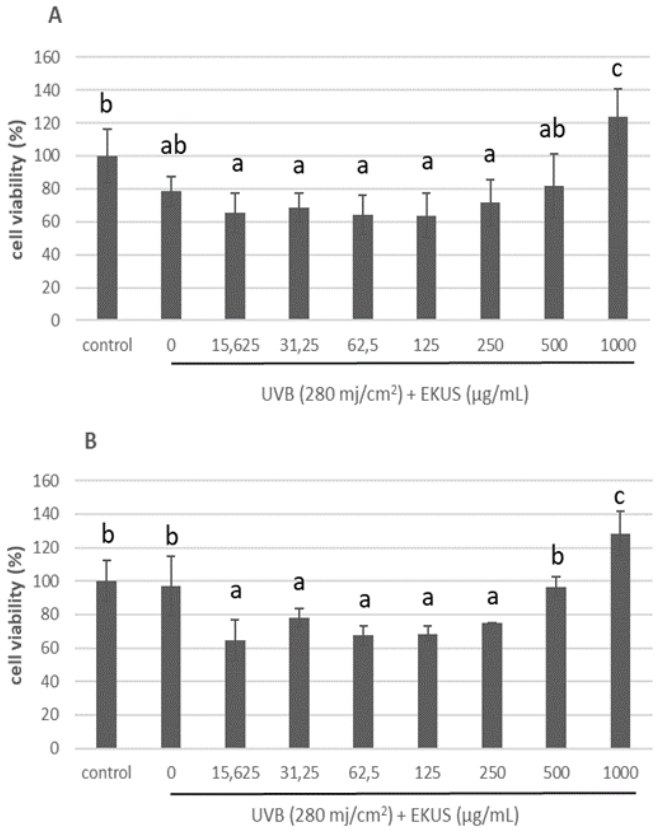

Figure 4. Cytoprotective effect of EKUS against UVB irradiation of 280 mJ cm^{-2} on HDF cells (A) cell density $5x10^3$ cells/well (B) density 10^3 cells/well.

Effect of EKUS on collagen production in UVB irradiated HDF cells

Collagen production is one of the parameters used to determine the protective effect of EKUS on HDF cell damage that is irradiated with UVB using Sirius red staining. Optimal collagen deposition with the right ratio is very important to prevent tissue damage during connective tissue formation (Su et al. 2019). HDF cells were incubated with EKUS for 48 hours, then exposed to UVB light at a dose of 280 mJ cm^{-2} for 344 s. UVB rays cause degradation of collagen synthesis, which causes skin ageing (Jung et al. 2014). In this study, UVB irradiation was able to reduce collagen deposition significantly ($p \leq 0.005$) compared to control cells. In fibroblast cells that only received UVB irradiation, the viability was only 58.08 ± 6.43 % (density $5x10^3$/well) and 62.60 ± 19.16 % (cell density 10^4/well). The results indicated that changes in EKUS concentration significantly impacted ($p \leq 0.005$) collagen deposition ratio in fibroblasts compared to cells that only received UVB irradiation (Figure 5).

EKUS treatment prior to UVB exposure on HDF cells ($5x10^3$ cells/well) began to increase collagen production significantly ($p \leq 0.005$) at concentration of ≥ 600 µg mL^{-1} (Figure 5A). Meanwhile, at a cell density of 10^4/well significantly at a concentration of ≥ 250 µg mL^{-1} (Figure 5B). The most effective EKUS treatment for enhancing collagen production is at a concentration of 900 µg mL^{-1}. This concentration significantly ($p \leq 0.005$) increased collagen production by <198 %. At a cell density of $5x10^3$/well the collage production value was 146.30 ± 27.20 % and at a cell density of 10^4/well the value was 189.04 ± 9.66 %. However, the effect started to decline to 20 % when reaching a concentration of 1000 µg mL^{-1} across two different cell densities (Figure 5). In line with research by Aramwit et al. (2010) silk sericin extracted using the high-heat method at concentrations of >200 µg mL^{-1} can induce collagen production in L929 mouse fibroblast cells after incubation for 24 hours. According to Aramwit et al. (2009), silk sericin enhances collagen

synthesis in fibroblast cells because of its amino acid composition, particularly methionine and cysteine. In experiments conducted by Su et al. (2019) on human fibroblasts, it was demonstrated that mulberry sericin (0.05 %) promotes cell proliferation and enhances the expression of type I and III collagen. Therefore, this study demonstrates that EKUS can protect HDF cells from UVB rays by increasing collagen production.

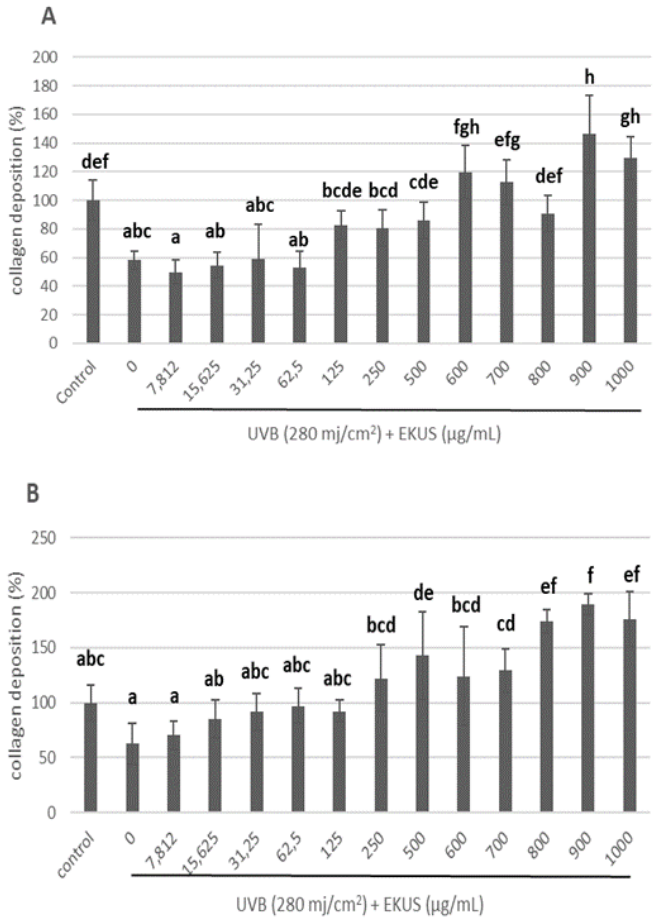

Figure 5. Effect of EKUS on collagen deposition in HDF cells exposed to 280 mJ cm $^{-2}$ UVB radiation (A) density 5x10^{3} cells/well (B) density 10^{4} cells/well.

CONCLUSION

EKUS exhibits protective effects on HDF cells against UVB irradiation by enhancing cell viability within a concentration range of 500 µg mL^{-1} to 1000 µg mL^{-1}. A concentration of 900 µg mL^{-1} of EKUS was found to maximise collagen production in UVB-exposed HDF cells, reaching up to <198 % of the untreated UVB group. Collagen is a key structural protein in the dermis, and its degradation is a hallmark of UV-induced skin damage. UVB radiation elevates levels of reactive oxygen species (ROS), triggering oxidative stress that disrupts fibroblast function and speeds up collagen degradation. EKUS, rich in sericin peptides, likely exerts its photoprotective effects through antioxidant activity that mitigates ROS-induced damage, preserves fibroblast viability, and supports collagen synthesis. By enhancing both cell survival and extracellular matrix integrity, EKUS shows promise as a potential therapeutic candidate for preventing UVB-induced skin ageing.

AUTHORS CONTRIBUTION

C.A. conceived and designed the experiment, conducted the procedures, collected and processed the data, and authored the manuscript. S.W., A.N., S.S. arranged, managed, and directed the experiment, made critical revisions, and endorsed the final version.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest from this manuscript.

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