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ORIGINAL ARTICLE

Protective Effects of *Centella asiatica* Leaf Extract Cream on IL-1α Expression and Sunburn Cells in BALB/c Mice Induced by UVB

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Abstract

Background/Aim: UVB radiation can induce DNA damage in keratinocyte cells, leading to increased levels of reactive oxygen species (ROS) triggered by the release of proinflammatory cytokine IL-1α and apoptosis (sunburn) of keratinocyte cells. *Centella asiatica* (*C asiatica*) leaf extract contains various secondary metabolites such as flavonoids, alkaloids, tannins and saponins, which have been proven to have antioxidant and anti-inflammatory activities. This study aimed to analyse the effect of *C asiatica* leaf extract cream on the expression of IL-1α and sunburn cells in acute UVB-induced BALB/c mice.

Methods: Experimental research was designed with a post-test control group of 4 groups: normal control group, negative control, 10 % *C asiatica* leaf extract cream group (P1) and 20 % *C asiatica* leaf extract cream group (P2). Each mouse was exposed to UV-B light broadband at a dose of 1 minimal erythema dose (MED) or energy 360 mJ/cm^2 from day 1 to day 5 for 6 minutes with a distance of 30 cm, while the standard group was not exposed to UVB. In P1 and P2, *C asiatica* leaf extract cream of 10 % and 20 % was applied daily for 5 days, while the negative control received base cream. On day 6, skin tissue samples were taken and analysis was performed for IL-1α expression using immunohistochemistry (IHC) and sunburn cells using haematoxylin and eosin staining.

Results: The expression of IL-1α in the treatment groups decreased with increasing doses, with P2 having IL-1α expression (43.33 ± 7.60), P1 (48.33 ± 8.33), negative control (69.17 ± 9.17) and standard control (50.00 ± 1.91). The number of sunburn cells also decreased in the P1 (1.08 ± 0.15) and P2 (1.20 ± 0.19) groups compared to the negative control group (1.48 ± 0.17).

Conclusion: The administration of *C asiatica* leaf extract cream can reduce the expression of the IL-1α gene and the number of sunburn cells in the skin tissue of mice induced by acute UVB radiation.

Key words: *Centella asiatica*; Interleukin-1alpha; Sunburn; Ultraviolet rays; UVB.

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Introduction

Ultraviolet B (UVB) radiation in the wavelength range of 280-320 nm is a major environmental factor that induces skin damage, such as sunburn and photodamage.[1, 2] Exposure to UVB rays leads to an elevated concentration of reactive oxygen species (ROS), which triggers inflammation and

activates pro-inflammatory cytokines like interleukin-1a (IL-1a).[3] The increased levels of IL-1a cytokines can initiate a secondary cascade of keratinocyte mediators, causing apoptosis through the induction of caspase-3 and caspase-9.[4] Additionally, UVB can induce apoptosis of keratinocytes, termed sunburn cells, *via* the clustering of apoptosis receptors, p53 and the CD95 pathway.[5, 6] Previous research has demonstrated that *Centella asiatica* (*C asiatica)* leaf extract at a dose of 200 mg/kg BW exhibits anti-inflammatory and antioxidant activities by reducing pro-inflammatory cytokines and ROS.[7] Furthermore, previous studies have reported that a 10 % *C asiatica* leaf extract cream exhibited the highest collagen levels and hydration increase compared to other creams ROS.[7]

Sunburn in humans occurs more frequently in individuals with lighter skin types and those residing closer to the equator, with approximately 50 % having a history of acute sunburn. In America, sunburn prevalence within one year is 13 % in African Americans and 30 % in Hispanic Americans.[8] A study conducted in 2015 involving 31,162 adult samples revealed that 34 % of participants had experienced at least one episode of UVB burn. Individuals with Fitzpatrick skin types I to III, characterised by lighter skin tones, those aged 18 to 29 and non-Hispanic white individuals exhibited a higher likelihood of experiencing sunburn when performing outdoor activities, with an annual UV radiation exposure of 30 % to 50 %. As a result, most of the population will develop darker skin due to relatively high UVB exposure, which initiates the process of inflammation, hyperpigmentation or photodamage. Approximately 40 %-50 % of women and 20 %-40 % of men aged 24-29 years suffer from photodamage. The prevalence of photodamage in adult women is around 70 % and in adult men it is around 60 %.[9]

Currently, the prevention of UVB exposure involves using various chemical and physical cosmetic ingredients to protect the skin from the effects of acute UVB exposure. However, the chemicals in cosmetics are aromatic compounds with carbonyl groups and can irritate, especially in individuals with sensitive skin. Therefore, using natural compounds is indispensable in addressing the problem of acute UVB exposure while minimising side effects.[10-12] The *C asiatica* plant has been extensively studied for its anti-inflammatory and antioxidant activities in various skin diseases, such as psoriasis and hyperpigmentation.[13] The anti-inflammatory mechanism of *C asiatica* is attributed to its active compounds, including flavonoids, terpenoids, alkaloids, tannins and saponins.[14] The flavonoid content in *C asiatica* has the potential to protect against sunlight due to the presence of a chromophore group with a conjugated aromatic system that can absorb UV A and UV B rays.[15] Previous research has reported that *C asiatica* leaf extract suppressed ROS with an IC_{50} value of 31.09 µg/mL *in vitro*.[16] *C asiatica* extract has also exhibited anti-inflammatory activity by suppressing the expression of pro-inflammatory cytokines by regulating the JAK/STAT3 pathway.[13]

Additionally, previous research has demonstrated that *C asiatica* protects against UVB-induced HaCaT keratinocyte damage through changes in microRNA expression.[17] The exploration of the potential of *C asiatica* leaf extract against photodamage due to UVB exposure remains unclear. Based on this background, the present study aimed to determine the effect of administering *C asiatica* leaf extract on the expression of IL-1 and sunburn cells in BALB/c mice induced by acute UVB radiation.

Methods

Extraction of *C asiatica* extract

C asiatica was collected from Semarang in Central Jawa, Indonesia, in April 2024. They were rinsed with tap water, followed by distilled water to remove the dirt on the surface. The dried *C asiatica* was blended until small pieces and sieved with a mesh size of 120 mesh. The 50 g of *C asiatica* was extracted in a maceration apparatus with 500 mL 98 % ethanol for 24 h. The filtrate was then evaporated under a rotary vacuum evaporator (IKA) and the crude extract was kept in refrigerator

Table 1: Cream formulation

Ingredients	Percentage (%)
Stearic acid	15.00
Cetyl alcohol	4.00
Triethanolamine	0.50
Glycerine	8.00
Methylparaben	0.10
Propylparaben	0.05
Distilled water	*ad* 100.00

4 °C.[18, 19] *C asiatica* extract (10 % and 20 %) was dissolved in cream bases. The formulations were stored 4 °C until further analysis.

Phytochemical analysis

Standard protocols were employed to analyse the crude *C asiatica* extract for flavonoids, alkaloids, tannins, steroids and saponins according to established methods.[20-24]

Quantification of total flavonoid content in *C asiatica* extract

Total flavonoid content was quantified *via* aluminium chloride colorimetric assay[25] with modifications. Gallic acid served as the reference standard, with a calibration curve constructed using concentrations ranging from 200-700 µg/mL. The assay mixture comprised 0.5 mL of extract or standard solution combined with 0.1 mL of 10 % aluminium chloride, 0.1 mL of 1 M potassium acetate, 1.5 mL of 80 % methanol and 2.8 mL of distilled water. The blank was prepared identically, substituting distilled water for sample/standard and aluminium chloride solutions. Following 30-minute incubation at room temperature, absorbance was measured at 415 nm using a UV/Vis spectrophotometer *(Shimadzu Corporation*, Japan). Results were expressed as mg gallic acid equivalent (GAE) per gram of extract.

Photodamage model

Twenty-four healthy male BALB/c mice (25 ± 2.5 g) CV = 10 % were fed *ad libitum* and reared at 28 ºC and a photoperiod of 12 h. After a week of acclimatisation, mice were randomly divided into the following four groups: healthy, UVB irradiation, UVB irradiation and 10 % cream of *C asiatica* extract and UVB irradiation and 20 % cream of *C asiatica* extract. Each group consisted of six rats. This study used UVB light (broadband with peak emission at 302 nm CL-100M, UVP, USA). Rats were exposed to UVB light of 390 mJ/cm^2 for 6 min for five consecutive days, according to a previous study with a slight modification.[26] The 100 mg of *C asiatica* extract creams were administered topically on the dorsal mice skin daily from day 1 until day 5. UVB group rats did not receive any treatment. On day 6, all mice were terminated and skin tissue was isolated for further analysis.

IL-1a expression by immunohistochemistry

The 5-µm sections were cut from paraffin-embedded tissues. For IHC staining of IL-1α, the sections were incubated with primary antibodies against IL-1α (1:100, *Abcam*, Cambridge, MA, USA) overnight at 4 °C. Thereafter, each section was incubated and visualised using the Dako REAL EnVision Detection System (*Agilent Technologies*, Santa Clara, CA, USA). Two investigators who were unaware of the clinical data examined the sections *via* light microscopy. The IHC score of IL-1α was analysed using the IHC Profiler plugin in *ImageJ* software.

Sunburn cells by haematoxylin and eosin staining

On the sixth day, the mice were euthanised and skin samples were collected. These samples were fixed in 10 % formaldehyde and embedded in paraffin. Each 5 mm thick skin section was stained with haematoxylin and eosin (H&E) using a standard protocol. Sunburn cells were identified and counted throughout the epidermis in each section *via* light microscopy. Identification of sunburn cells was based on their morphological characteristics, such as cell membrane shrinkage and nuclear condensation resulting from cellular fragmentation. The sunburn cells were enumerated across the epidermis of each section using a 1 cm by 1 cm grid inserted into a conventional microscope. Counts were conducted over an entire 1.0 cm long epidermal section. Data are reported as the number of sunburn cells/cm of epidermal length, with a sample size of n = 6 per group.

Statistical analysis

The statistical significance of the differences between the treatment and control groups was assessed using one-way ANOVA, followed by the post hoc Tamhane's test. This analysis was conducted with *GraphPad Prism* version 4.00 for Windows (*GraphPad Software*, San Diego, California, USA). A p-value of less than 0.05 was considered statistically significant.

Results

The extract of *C asiatica* used in this study was prepared through the maceration method, utilising ethanol as the solvent, which resulted in an extract yield of 6.54 %. Phytochemical screening indicated that the *C asiatica* extract contained various compounds, including phenols, phenolic acids, tannins, flavonoids, alkaloids and saponins (Table 2). Additionally, spectrophotometric tech-

niques were employed to assess the total flavonoid and phenolic content in the extract. Specifically, one gram of *C asiatica* extract was found to contain 70.16 mg ± 1.22 of flavonoids and 45.80 mg ± 1.65 of phenolics. These findings suggest that a significant portion of the compounds present in the *C asiatica* extract were flavonoids.

This study examined the effects of *C asiatica* leaf extract cream on the expression of IL-1α and sunburn cells in male BALB/c mice exposed to acute

Table 2: Phytochemical screening of secondary metabolites from C asiatica extract

Chemical component	Test	*C asiatica* extract
Alkaloids	Wagner test	+
Flavonoids	Wilstater test	+
Tannins	Braemer's test and Keller-Killiani test	+
Saponins	Forth test	+
Steroids	Lieberman Burchardt test	-

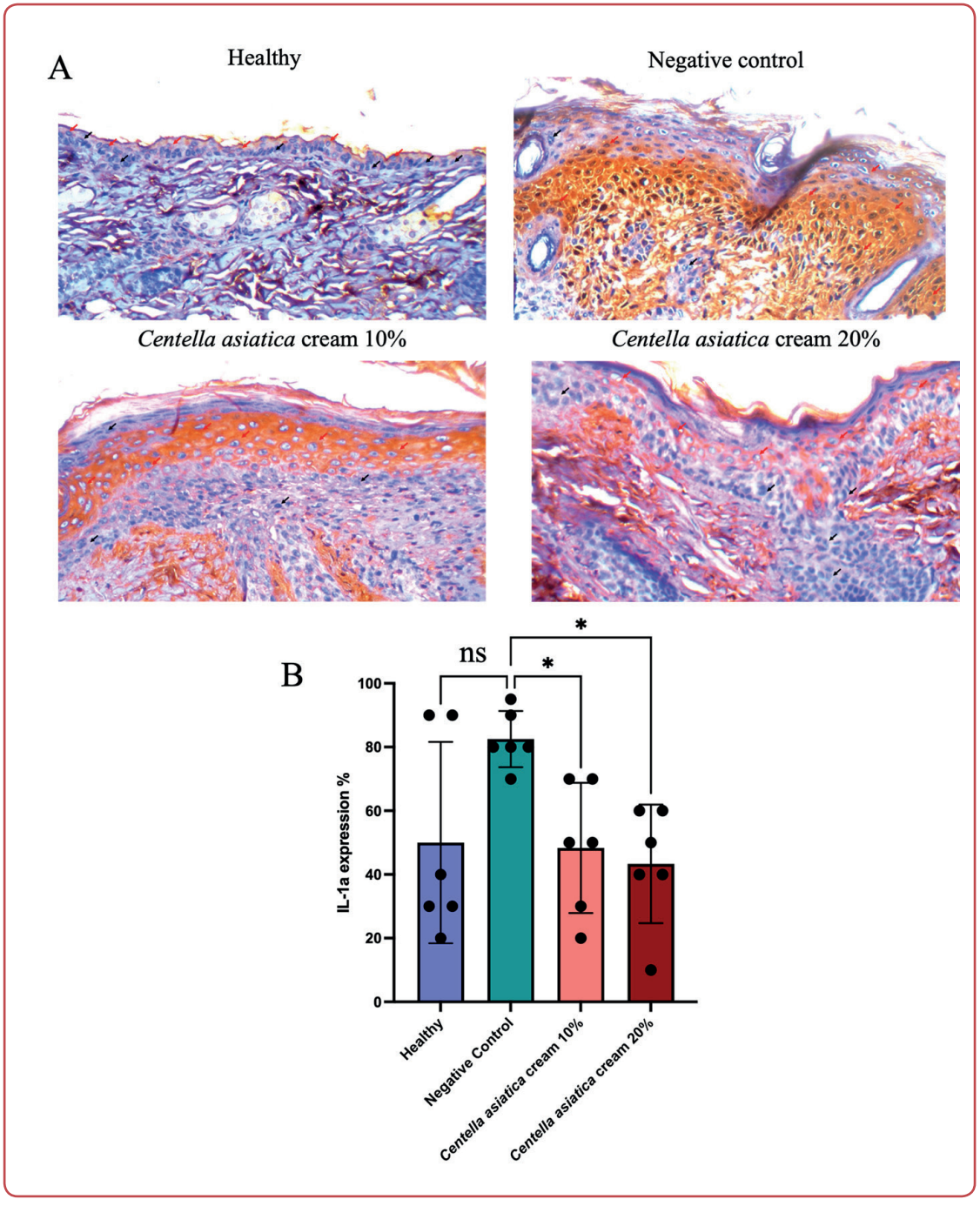

*Figure 1: (A) Histopathological depiction of interleukin 1 alpha (IL-1α) expression with immunohistochemistry (IHC) staining. (B) Graph of IL-1α expression across all study groups. *: p < 0.05; Arrows indicate positive IL-1α expression; n = 6;*

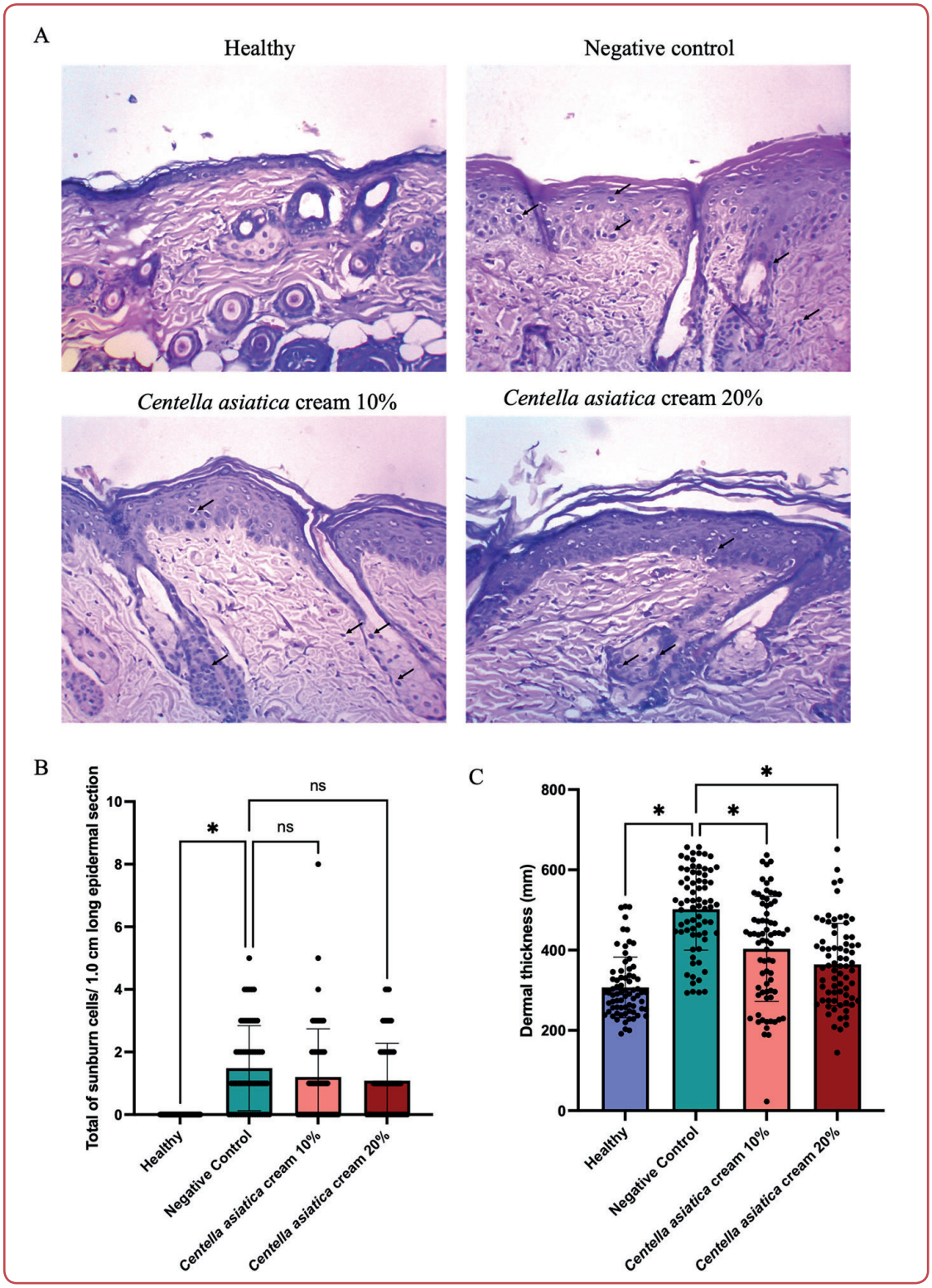

*Figure 2: (A) Histopathological depiction of sunburn cells with haematoxylin and eosin (H&E) staining. (B) Graph of sunburn cells across all study groups. (C) Dermal thickness. *: $p < 0.05$; ns: $p > 0.05$; arrows indicate sunburn cells;*

UVB radiation. In this research, the expression of IL-1α was analysed using the IHC method on the 6th day, one day after the last administration of *C asiatica* extract cream. The mean expression of IL-1α was lowest in the 20 % *C asiatica* extract cream group (43.33 ± 7.60), followed by the 10 % *C asiatica* extract cream group (48.33 ± 8.33). The highest IL-1α expression was observed in the negative control treatment group, with a mean of 82.50 ± 3.59 (Figure 1A-B).

C asiatica leaf extract cream reduced the number of sunburn cells in BALB/c mice exposed to acute UVB radiation (Figure 2A-B). The highest mean number of sunburn cells was in the negative control group (1.48 ± 0.17), followed by the 10 % *C asiatica* extract cream group (1.20 ± 0.19) and the 20 % *C asiatica* extract cream group (1.08 ± 0.15). The normal control group did not show any sunburn cells. This study also found that administration of 10 % and 20 % *C asiatica* leaf extract significantly reduced dermal thickness, preventing dermal damage caused by UVB exposure (Figure 2C).

Discussion

UVB radiation is a significant cause of skin photodamage, characterised by excessive inflammation.[21] The inflammatory response induced by UVB and photoaging of the skin is caused by an increase in the production of ROS, thereby activating the NF-κB signalling pathway in the epidermis, which leads to an increase in pro-inflammatory cytokines such as IL-1α.[2, 22] Previous research has reported that the rise in ROS will activate MDA, which will induce the NF-κB transcription factor and release pro-inflammatory cytokines that play a crucial role in the maintenance and expansion of skin damage characterised by sunburn.[23, 24] This suggests that suppressing pro-inflammatory cytokines and reducing the number of sunburn cells is expected to inhibit the photodamage induced by acute UVB radiation. This study investigated the effect of administering 10 % and 20 % *C asiatica* extract cream on the expression of IL-1α and the number of sunburn cells in BALB/c mice induced by acute UVB radiation.

This study analysed the expression of IL-1α and the number of sunburn cells in BALB/c mice induced by acute UVB radiation. The *C asiatica* extract cream contains secondary metabolite compounds such as flavonoids, alkaloids, tannins and saponins. The presence of these secondary metabolite compounds can suppress the balance of ROS levels by increasing the formation of the antioxidant system, thereby inhibiting inflammation through the inhibition of NF-κB activation.[25-28] The secondary metabolite content in *C asiatica* leaves has also exhibited anti-inflammatory activity by suppressing the expression of IL-1α. Previous research has reported that flavonoid and phenolic compounds can induce IL-10, which plays a role in the suppression of inflammation through the activation of several intracellular proteins, one of which is the Suppressor of cytokine signalling 3 (SOSC3).[29, 30] IL-10 will bind to the receptor and activate the signal transducer and activator of the transcription 3 (STAT-3) signalling pathway. The STAT3 protein will enter the nucleus and activate the SOSC3 mRNA sequence. The SOSC3 protein is then expressed intracellularly and suppresses various pro-inflammatory signalling pathways, including NF-κB.[31, 32] Suppressing the NF-κB pathway will decrease the secretion of various pro-inflammatory cytokines, such as IL-1α, which will inhibit the process of keratinocyte cell apoptosis.[33] Previous research has reported that flavonoid compounds such as luteolin-7-sulfate, also present in *C asiatica* extract, suppress sunburn in keratinocyte cells.[15, 17]

In this study, the reduction in IL-1α expression significantly differed among all treatment groups. *C asiatica* extract inhibits the death or sunburn of keratinocyte cells by targeting p63-miRNA, which involves the MAPK pathway.[17, 34] The MAPK-mediated signalling pathway is reportedly engaged in the UVB response in keratinocyte sunburn.[34] In this study, the *C asiatica* extract cream was proven to reduce the number of sunburn cells in a dose-dependent manner. These results support previous research findings that reported *C asiatica* extract inhibits UVB-induced apoptosis in HaCaT keratinocytes by modulating the expression of apoptosis-related genes such as Bcl-2 and Bax.[17] *C asiatica* extract can also reduce ROS levels and lipid peroxidation in keratinocytes exposed to UVB radiation, thereby reducing oxidative stress, which can inhibit sunburn.[35, 36] Previous research has reported that treatment with troxerutin, a flavonoid from *C asiatica* leaves, alters the expression of 23 miRNAs in human dermal fibroblasts exposed to UVB.[17, 34] These miRNAs target genes involved in cell cycle regulation, apoptosis and DNA repair pathways, contributing to the protective effect against UVB irradiation. Troxerutin also increases DNA repair activity in human dermal fibroblasts exposed to UVB, potentially by regulating the expression of miRNAs targeting DNA repair genes, thereby inhibiting sunburn. *C asiatica* extract significantly reduces the expression of pro-inflammatory cytokines such as IL-6 and TNF-α in keratinocytes exposed to UVB radiation,[15, 16, 34] demonstrating anti-inflammatory properties, which is also similar to the results of this study where *C asiatica* extract suppresses IL-1α, thereby reducing the number of sunburn cells.

The optimal reduction in IL-1α expression and sunburn cells in this study was achieved by the 20 % *C asiatica* extract cream. The decrease in IL-1α will inhibit the STAT and MAPK pathways, thereby preventing DNA damage in keratinocyte cells and sunburn.[37, 38] A limitation of this study is the lack of examination of ROS levels and exploration of the NF-κB pathway after the application of *C asiatica* extract cream, so the direct molecular mechanism of the extract in preventing photodamage is still unclear. In this study, only short-term UVB exposure was performed, so longer exposure or a higher MED dose that significantly increases the massive release of pro-inflammatory cytokines is required.

Conclusion

The results of this study indicate that *C asiatica* extract cream exhibits anti-inflammatory activity in a mouse model exposed to acute UVB radiation through the suppression of IL-1α expression and the number of sunburn cells. This suggests that *C asiatica* extract cream has the potential to be developed as a targeted therapy to suppress the inflammatory process, particularly in photodamage.

Ethics

The study was approved by the Ethics Committee of the Medical Faculty, Universitas Islam Sultan Agung, Semarang, Indonesia, under Decision No 32/I/2024/Komisi Bioetik, dated 30 January 2024.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

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Writing - review and editing: PM, PS
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