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The effect of butterfly pea flower (*Clitoria ternatea*) extract gel on TNF-α and caspase-3 expression in wound tissue of wistar rats

Pengaruh pemberian gel ekstrak bunga telang (Clitoria ternatea) terhadap ekspresi TNF-α dan caspase 3 pada jaringan luka tikus wistar

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Abstract

Exposure to acute ultraviolet B (UVB) damages the skin through oxidative stress, inflammation, and apoptosis. UVB increases the production of reactive oxygen species (ROS), which damage cells and trigger inflammation by increasing TNF-α levels. Increased ROS and TNF-α levels activate caspase-3, which causes cellular apoptosis. This damage worsens skin conditions, triggers premature aging (photoaging), and increases the risk of skin cancer. In Indonesia, approximately 57,3% of the population is exposed to sunlight, with a prevalence of dry skin ranging from 50% to 80%, while photoaging contributes to approximately 80% of the adverse effects of skin aging. Clitoria ternatea contains bioactive compounds with antioxidant and anti-inflammatory properties that have the potential to suppress caspase-3 activation and prevent cell damage. This experimental study used a post-test-only control group design with a completely randomized design, involving male Wistar rats divided into four treatment groups (K1–K4). The study was conducted at the Chemistry Laboratory of IBL UNISSULA, with animal treatment at the Animal Experiment Laboratory of IBL UNISSULA and skin tissue sample analysis at the General Medical Laboratory of CITO Yogyakarta from December 2024 to February 2025. Clitoria ternatea extract gel was applied topically once a day for 7 days, followed by UVB exposure (160 mJ/cm^2) for ±15 min per day. Skin tissue samples were collected 24 h after the last treatment with a 6 mm punch biopsy, and the expression of TNF-α and caspase-3 was analyzed using qRT-PCR. Data were tested using one-way analysis of variance (ANOVA). Results, the group given a 5% dose of gel (K3) showed a significant decrease in the expression of TNF-α (0,35±0,20) and caspase-3 (0,16±0,32) compared to other groups, while the 10% dose (K4) showed no significant difference. The conclusion, Clitoria ternatea extract gel at a 5% dose effectively reduced the expression of TNF-α and caspase-3 in the skin of UVB-exposed mice.

Keywords: Clitoria ternatea, TNF-α, caspase-3, UVB, apoptosis

Abstrak

Paparan sinar ultraviolet B (UVB) akut merusak kulit melalui stres oksidatif, peradangan, dan apoptosis sel. UVB meningkatkan produksi reactive oxygen species (ROS), yang merusak sel dan memicu peradangan dengan meningkatkan TNF-α. Peningkatan ROS dan TNF-α kemudian mengaktifkan caspase-3, yang menyebabkan apoptosis seluler. Kerusakan ini memperburuk kondisi kulit, memicu penuaan dini (photoaging), dan dapat meningkatkan risiko kanker kulit. Di Indonesia, sekitar 57,3% penduduk terpapar sinar matahari, dengan prevalensi kulit kering yang berkisar antara 50% hingga 80%, sementara photoaging berkontribusi sekitar 80% terhadap efek buruk penuaan kulit. Clitoria ternatea mengandung senyawa bioaktif dengan sifat antioksidan dan anti-inflamasi yang berpotensi menekan aktivasi caspase-3 dan mencegah kerusakan sel. Penelitian eksperimental ini

menggunakan desain post-test only control group dengan rancangan acak lengkap, melibatkan tikus jantan galur Wistar yang dibagi dalam empat kelompok perlakuan (K1–K4). Penelitian dilakukan di Laboratorium Bagian Kimia IBL UNISSULA, dengan perlakuan hewan di Laboratorium Hewan Coba IBL UNISSULA dan analisis sampel jaringan kulit di Laboratorium Medis Umum CITO Yogyakarta, selama Desember 2024–Februari 2025. Gel ekstrak *Clitoria ternatea* diberikan secara topikal sekali sehari selama 7 hari, diikuti paparan UVB (160 mJ/cm^2) selama ±15 menit per hari. Sampel jaringan kulit diambil 24 jam pasca perlakuan terakhir dengan biopsi punch 6 mm, dan ekspresi TNF-α serta caspase-3 dianalisis menggunakan qRT-PCR. Data diuji menggunakan One-Way ANOVA. Hasil, kelompok yang diberikan gel dosis 5% (K3) menunjukkan penurunan signifikan pada ekspresi TNF-α (0,35±0,20) dan caspase-3 (0,16±0,32) dibandingkan kelompok lain, sedangkan dosis 10% (K4) tidak menunjukkan perbedaan bermakna. Kesimpulan, gel ekstrak *Clitoria ternatea* dosis 5% efektif menurunkan ekspresi TNF-α dan caspase-3 pada kulit tikus yang terpapar UVB.

Kata Kunci: Klitoria ternatea, TNF-α, caspase-3, UVB, apoptosis

Introduction

Acute exposure to ultraviolet B (UVB) rays not only causes erythema but also triggers various negative effects on the skin, including oxidative stress, DNA damage, inflammation, and cellular apoptosis (Na & Ryu, 2018). An increase in ROS (*Reactive Oxygen Species*) as a trigger for inflammation causes neutrophil-type leukocytes to secrete pro-inflammatory proteases and cytokines such as *Tumor Necrosis Factor alpha* (TNF-α) (Na & Ryu, 2018). TNF-α causes apoptosis by activating caspase-3 which then degrades the proteins present (Muhartono & Subeki, 2017). Telang flower (*Clitoria ternatea*) with its bioactive content is able to interfere with TNF-α triggered pathways to reduce Caspase-3 activation, thereby preventing protein degradation and excessive cell damage (Puspitasari et al., 2019; Widiyanto et al., 2022). Telang flower extract is rich in anthocyanins, flavonoids, and phenolic compounds with antioxidant and anti-inflammatory properties (Marpaung, 2020).

Innovation opportunities in the development of natural ingredient-based gel preparations as a safer, more economical, and environmentally friendly alternative to synthetic products, with a focus on the effects of gels on the expression of TNF-α and Caspase-3, as well as paving the way for innovation in herb-based dermatological therapies (Garcella et al., 2023). Previous research confirms the scientific urgency of this study, using telang flower gel extract significantly reduced UVB-induced TNF-α and caspase-3 production in mice for 14 days (Cahyani et al., 2023). The focus of this study is on the effect of telang flower gel on the expression of TNF-α and Caspase-3, which will be carried out for 7 days. This study further confirms its scientific urgency, while paving the way for innovation in herb-based dermatological therapies (Garcella et al., 2023).

Tumor necrosis factor-alpha (TNF-α) is a cytokine with pleiotropic effects on various cell types. TNF-α has been identified as a key regulator of the inflammatory response and is involved in the pathogenesis of several inflammatory and autoimmune diseases. Structurally, TNF-α is a homotrimeric protein composed of 157 amino acids, mainly produced by activated macrophages, which are functionally known to trigger a series of inflammatory molecules, including cytokines and other chemokines. TNF- α exists in soluble and transmembrane forms (Jang et al., 2021). TNF-α can trigger apoptosis by activating caspase-3 which then degrades existing proteins. Caspase-3 is an executor caspase class activated by initiator caspases such as caspase-8 and caspase-9. Activation of apoptosis in the extrinsic and intrinsic pathways leads to the activation of caspase-3 as an executor caspase. If caspase-3 has been activated, the apoptosis process will occur (Muhartono & Subeki, 2017).

Overproduction of ROS increases inflammation, characterized by the release of various pro-inflammatory molecules, such as TNF-α. UV irradiation converts dermal collagen through collagen breakdown pathways (matrix metalloproteinases [MMPs]) and inhibits the inflammatory process. The procollagen synthesis pathway results in loss of collagen content. UV-induced ROS damage DNA and induce lipid peroxidation and protein degradation in skin cells. In addition, ROS reduce

the activity of antioxidant enzymes in the skin, including superoxide dismutase and glutathione peroxidase. Prolonged production of TNF-a induces ROS which causes oxidative damage to DNA via the NADPH-oxidase 1,5 pathway thereby activating the p53 gene, which leads to caspase-3 expression and triggers apoptosis in skin cells, including fibroblasts (Cahyani et al., 2023).

Phenolic compounds, ascorbic acid and carotenoids, which are derived from plant species, are able to protect the skin by preventing the penetration of UV rays, reducing inflammation and oxidative stress (Petruk et al., 2018). At this time, many natural ingredients have been developed for faster and more effective wound healing, one of which is the telang flower (Sagala et al., 2016).

Clitoria ternatea is a plant with a lot of nutritional content and is known as a medicinal plant in various parts of the world (Oguis et al., 2019). All parts of the plant including roots, seeds and leaves, are used as medicines and are recognized to have various effects such as effects in lowering inflammation (Kumar et al., 2017; Syafa'Atullah et al., 2020). The crown of the telang flower is a source of anthocyanins and various types of flavonoids that have antioxidant effects, anthocyanins are powerful antioxidants that can lower ROS which can be given topically or orally (Xiang et al., 2017).

Recent research reports that telang flower extract cream has high levels of antioxidants that are able to inhibit ROS production and reduce inflammatory conditions so that it can inhibit the increase in MMP, prevent fibroblast cell apoptosis and inhibit collagen decline (Jayanti et al., 2021; Saritani et al., 2021). Another study also reported that the use of *Clitoria ternatea L* 5% extract gel was shown to inhibit the increase in MMP-1 expression in the skin of Wistar rats exposed to UV-B rays (Subchan et al., 2022).

He aim of this study is to evaluate and compare the effects of topical application of 5% and 10% Telang (Clitoria ternatea) extract gel on the expression of TNF-α and Caspase-3 in the skin tissues of Wistar rats exposed to UV-B radiation.

Methods

This experimental research used *a post-test control group design* with a complete random design method conducted at the IBL Unissula Chemistry Laboratory, while the treatment of experimental animals was carried out at the IBL Unissula Laboratory, and skin tissue samples were analyzed at the CITO Yogyakarta General Medical Laboratory. The study was conducted from December 2024-February to 2025. The subjects used in this study were male Wistar rats (Rattus norvegicus) that were declared healthy and suitable for use in research, and were exposed to 302 nm UVB light at a distance of 20 cm with MED 160 mJ/cm2/day. The research sample consisted of 20 mice that were divided into four treatment groups: K1 (healthy mice were not exposed to UV-B), K2 (control, mice were given a gel base and exposed to UV-B), K3 (Treatment 1, mice were given a 5% dose of Clitoria ternatea L extract gel and exposed to UV-B), and K4 (Treatment 2, mice were given a 10% Clitoria ternatea L extract gel and exposed to UV-B).

Ethical Clearance

The application for the ethical clearance of research was submitted to the Ethics Commission of the Faculty of Medicine, Sultan Agung Islamic University, Semarang. NO.23/I/2025/Komisi Bioetik

How to Make Telang Flower Extract

Telang flowers (250 g) were washed and dried at 40°C to reduce the moisture content. After drying, the telang flowers were ground using a grinding tool until they became a fine powder. The dry powder was then extracted using the maceration method with 1000 ml of 96% ethanol solvent for three days, with periodic stirring to ensure optimal extraction. After the first maceration process was completed, the mixture was filtered to separate the filtrate from the residue. The remaining residue was then reamacrated using the same method to obtain the maximum extraction results. The filtrate obtained was collected, and the solvent was evaporated using a rotary evaporator until a thick extract was formed. From this process, a thick extract weighing 40,1 grams was obtained with a yield of 16,04%, which was calculated based on the comparison between the weight of the extract obtained and the amount of initial powder multiplied by 100%. The resulting viscous extract was stored in a refrigerator at 2–8°C to maintain its stability and quality.

How to Make Telang Flower Extract Gel

The manufacture of Telang flower extract gel preparations begins by weighing all the

necessary ingredients. HPMC weighing 6,5 grams is developed in aquadest by stirring and then left overnight until it expands completely. Meanwhile, 0,02 grams of methyl paraben and an additional 0,18 grams of methyl paraben were dissolved in propylene glycol (10 g), liquid paraffin, and 1,08 grams of Tween 80. This mixture was then stirred until homogeneous before being added to the HPMC, which had expanded, and then stirred again until a gel base was formed. For the manufacture of a 5% dose of telang flower extract gel preparation, as much as 1 gram of extract is mixed with 19 grams of gel base, resulting in a total of 20 grams of preparation, while for a 10% dose, as much as 2 grams of extract is mixed with 18 grams of gel base so that it produces the same total preparation, which is 20 grams.

UVB Irradiation and Treatment of Experimental Subjects

The mice used in this study were adapted for five days after arriving at the study site. After the adaptation period, the hair on the backs of the mice was shaved clean with an area size of 3 × 4 cm to ensure that the gel application area and UVB exposure were evenly distributed. Rats in the Treatment 1 group were given topical telang flower extract gel at a concentration of 5%, while the treatment 2 group was given topical telang flower extract gel at a concentration of 10%. The gel was applied once a day for 7 days, and 30 min after application, the backs of the rats were exposed to UVB light from a distance of 20 cm using a minimum erythema dose (MED) of 160 mJ/cm^2 for ±15 min per day. This exposure causes erythema in the backs of mice due to the cumulative effect of UVB absorption in the epidermis. On the 8th day, termination was performed for further analysis.

Tissue Sampling

After 24 hours after the last treatment, mice were euthanized using the cervical dislocation method for tissue sampling. Skin tissue was taken by a 6 mm punch biopsy on the UVB-exposed area, stored in a tube containing RNA, and placed in a freezer at -80°C until qRT-PCR analysis was performed. RNA isolation begins by cutting 10–30 mg of skin tissue from each sample. The tissue was then removed using a tissue grinder in liquid nitrogen and transferred to an RNAse-free tube. Next, 0,3 mL of Binding Buffer 4 (which has been mixed with β-mercaptoethanol) and 15 µL of proteinase K for every 10 mg of tissue were added, followed by homogenization using a vortex. The mixture was incubated for 10–20 minutes at 56°C to ensure optimal cell lysis. After incubation, the sample was centrifuged at 12,000 × g for 5 min at room temperature and the resulting supernatant was transferred to a new RNAse-free tube for further analysis.

DNA Extraction and PCR Analysis

The gene expression test process begins with preparing RNA from each sample and a housekeeping gene (β-actin) as a comparison control. Next, a master mix was prepared with a composition that included 2x PerfectStart Green One-Step qRT-PCR SuperMix of 10 µL, forward primer (10 µM) and reverse primer (10 µM) of 0,4 µL each, TransScript® RT/RI Enzyme Mix 0,4 µL, Passive Reference Dye (50x) of 0,4 µL, sample RNA of 5 µL, and RNA se-free water of 3,4 µL, so that the total volume in each reaction reached 20 µL. After all components were mixed, there were no air bubbles in the mixture. The master mix was then inserted into each well on a strip tube, homogenized using a vortex, and a spindle was used before PCR began. The PCR program was set up as follows: reverse transcription at 50°C for 5–15 min (one cycle), RT, and DNA denaturation at 94°C for 30 s (one cycle), followed by PCR amplification at 94°C for 5 s and annealing and extension at 58°C for 30 s, which lasted for 45 cycles. SYBR Green fluorescence was detected during the annealing and extension stages. Finally, the sample in the tube strip was inserted into the PCR device and the program was run according to the predetermined time to obtain the desired gene expression data.

TNF-α and Caspase 3 Expression Analysis

The amplification curve shows a consistent exponential pattern in the absence of anomalies. Relative gene expression analysis was carried out using the ΔΔCt method by comparing the Ct value of the experimental sample against the housekeeping gene (β-actin) as an internal control.

Data from the analysis of TNFα and caspase expression were tested for normality and data variation using the Shapiro-Wilk test and Levene's test. The data results were normal ($p>0,05$) and homogeneous ($P>0,05$) for TNFα expression, so a one-way ANOVA test was performed. followed by the post-hoc LSD test to determine the significance of the differences between the two research groups.

Result and Discussion

This experimental study aimed to determine the effect of *Clitoria ternatea* extract gel on the expression of TNF-α and Caspase-3 in the skin exposed to acute UV-B rays. The research design used was a post-test-only control group with a complete random design method, involving four treatment groups. The subjects of the study were male Wistar rats (*Rattus norvegicus*), 20 2–3 months old with a body weight of 200–250 g. Mice that met the inclusion and exclusion criteria were adapted for seven days, then randomly divided into four groups: group 1 (healthy mice without UV-B exposure), control group 2 (mice administered a placebo gel followed by UV-B exposure), group 3 (mice administered a 5% *Clitoria ternatea* extract gel followed by UV-B exposure), and group 4 (mice administered a 10% Clitoria ternatea extract gel after exposure to UV-B). The hair on each rat on the back was shaved clean with a size of 3×4 cm, then administered topically according to the group, after 30 minutes of exposure to UV-B at a distance of 20 cm with a minimum *erythema dose* (MED) of 160 mJ/cm^2 for ±15 minutes per day for seven days.

The extraction *of Clitoria was carried* out in the Chemistry Laboratory of IBL Unissula, while the experimental animals were treated in the IBL Unissula Laboratory of Experimental Animals. Skin tissue samples were analyzed at the CITO Yogyakarta General Medical Laboratory using the Polymerase Chain Reaction (PCR) biomarker detection method with the Bio-Rad CFX 96 tool. Prior to sample testing, manual extraction and measurement of genetic material concentrations were performed using qubits. The expression of TNF-α and Caspase-3 was measured using β-actin as an internal control.

Results of analysis of TNF-α expression of skin tissue in each group

The results of the analysis of the average expression of TNF-α in the skin tissue of the mice were given topically based on the treatment of each group and then exposed to acute UVB light. The rat group (K1) was 0,85±0,15, the rat group that was given a gel and exposed to UV-B (K2) at 0,78±0,32, the rat group was given a 5% *dose of Clitoria ternatea L* extract gel and exposed to UV-B (K3) at 0,35±0,20, and the rat group was administered a 10% dose of *Clitoria ternatea L* extract gel and exposed to UV-B (K4) at 0,54±0,30, as shown in Table 1.

Table 1. Results of the average description of TNF-α expression

Group	K1 N=5	K2 N=5	K3 N=5	K4 N=5	p value
Ekspresi Gen TNF-α (ΔΔCq)					
Mean	0,85	0,78	0,35	0,54	
SD	±0,15	±0,32	±0,20	±0,30	
Shapiro-Wilk	0,393	0,332	0,062	0,672	
Leuvene Test					0,304
One way anova					0,024
Ekspresi gen caspase 3 (ΔΔCq)					
Mean	0,83	0,89	1,60	1,52	
SD	±0,11	±0,38	±0,32	0,50	
Shapiro-Wilk	0,783	0,272	0,141	0,277	
Leuvene Test					0,051
One way anova					0,005

Shapiro-Wilk = Normal (p>0,05); Levene Test = Homogeneous (p>0,05); Oneway anova = Significant (p<0,05)

The average results of the Shapiro-Wilk normality test on the expression of TNF-α showed that all groups were normally distributed, with a value of p>0,05, in the K1, K2, K3, and K4 groups, while the results of the homogeneity test with *the Levene Test* showed a value of p=0,304 (p>0,05), which means that the data had a homogeneous variance. The mean TNF-α expression was normally distributed, and the data variation was homogeneous so that the one-way ANOVA parametric statistical test could be carried out with a value of p = 0,024 (p<0,05); thus, there was a significant difference between all treatment groups for TNF-α expression.

The significant *results of the One Way Anova* test were followed by a test of the difference between the two groups with *the Post Hoc LSD* test to find out which group pairs were different.

Table 1. Post Hoc LSD *Test* TNF-α and caspase 3 Expression

Group	Mean/SD	Comparison Group	sig	95% Confidence Interval	
				Lower Bound	Upper Bound
TNF-α Expression					
K1	0,85±0,15	K1-K2	0,659	-,2679	,4119
K2	0,78±0,32	K1-K3	0,006	,1621	,8419
K3	0,35±0,20	K1-K4	0,068	-,0259	,6539
K4	0,54±0,30	K2-K3	0,016	-,2679	,4119
		K2-K4	0,151	-,0979	,5819
		K3-K4	0,259	-,5279	,1519
Caspase 3 Expression					
K1	0,85±0,15	K1-K2	0,999	-,8375	,6935
K2	0,78±0,32	K1-K3	0,022	-1,410	-,1377
K3	0,35±0,20	K1-K4	0,196	-1,737	,3533
K4	0,54±0,30	K2-K3	0,079	-1,4749	,0709
		K2-K4	0,317	-1,6203	,3803
		K3-K4	1,000	-,8966	1,0606

Description: Different meanings p<0,05

Based on the results of the comparison of the two groups on TNF-α expression, the pairs of groups that had the same differences were groups K1, K3, and K2 with K3, while the pairs of groups that did not differ were groups K1 with K2, K1 with K4, K2 with K4, and K3 with K4. While the results of the comparison of the two groups on caspase 3 expression showed that the pairs of groups that had differences were only groups K1 and K3, while the pairs of groups that did not differ were groups K1 with K2, K1 with K4, K2 with K3, and K4.

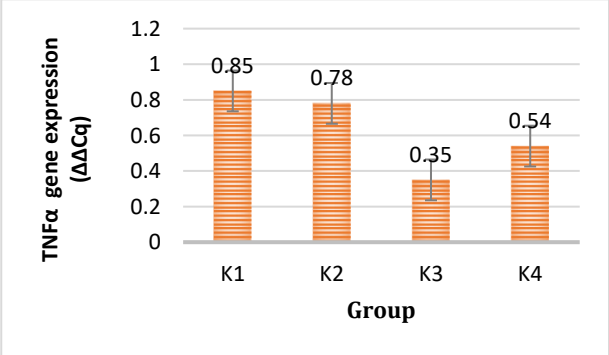

Figure 1. Graph of mean TNF-α expression of each treatment group

The results of the mean analysis (Figure 1) showed that the expression of TNF-α in the skin tissue of mice varied according to the treatment. The healthy rat group without UV-B (K1) exposure had an average TNF-α expression of 0,85 ± 0,15, while the group of mice given a gel base without Clitoria ternatea extract and exposed to UV-B (K2) had a slightly lower expression, which was 0,78 ± 0,32. The absence of a significant difference between K1 and K2 suggests that UV-B exposure does not significantly increase TNF-α expression in these conditions, or that the effects of the gel base may help maintain expression levels that are almost the same as normal conditions (Cahyani et al., 2023).

Administration of a 5% dose of *Clitoria ternatea* extract gel before UV-B (K3) exposure led to a significant decrease in TNF-α expression to 0,35 ± 0,20. Significant differences between K1 and K3, as well as K2 and K3, suggest that a 5% dose *of Clitoria ternatea* extract has a pronounced protective effect by decreasing the expression of TNF-α, a biomarker of inflammation due to UV-B exposure.

The group administered a 10% dose of *Clitoria ternatea* extract gel (K4) showed TNF-α expression of 0,54 ± 0,30. Although lower than K1 and K2, there were no significant differences between K1 and K4, K2 and K4, or K3 and K4. This indicates that increasing the extract dose from 5% to 10% did not have a significant effect in suppressing TNF-α expression. Overall, these findings suggest that a 5% dose is more effective at lowering TNF-α expression than a 10% dose, which may be due to an optimal effect at moderate doses, whereas higher doses may lead to a compensatory mechanism or mild stimulation effect on TNF-α expression. The absence of significant differences between K3 and K4 also suggested that increased doses are

not necessarily directly proportional to greater anti-inflammatory effects.

Results of analysis of the expression of 3 skin tissue caspases in each group

The results of the analysis of the average expression of caspase 3 in the skin tissue of rats given topically according to group treatment and after exposure to acute UVB rays were obtained in the healthy rat group (K1) at baseline 0,83±0,11, the rat group that was given a gel base and exposed to UV-B (K2) was 0,89±0,38, the rat group was given *Clitoria ternatea L* extract gel a dose of 5% and exposure to UV-B (K3) of 0,160±0,32, and a group of rats given a dose of *Clitoria ternatea L extract gel* at a dose of 10% and exposed to UV-B (K4) of 1,52±0,50, can be seen in table 1.

The average results of caspase 3 expression were tested for data normality with *Shapiro-Wilk* obtained for all groups with normal distribution, with a value of p>0,05, and the results of the homogeneity test with *the Levene Test* showed a value of p=0,041 (p>0,05), which means that the data had a non-homogeneous variance. The average expression of caspase 3 was normally distributed and the variation of data was not homogeneous; therefore, the parametric statistical test *of one-way ANOVA* could be carried out with a value *of p* 0,005 (p<0,05), and it was concluded that there was a significant difference between all treatment groups for the expression of caspase 3.

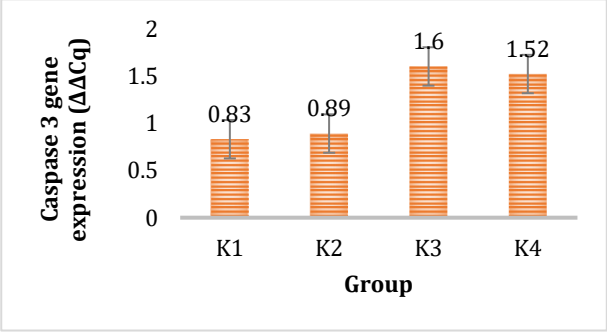

Figure 1. Graph of the average expression of caspace 3 for each treatment group

The results of the analysis showed that the expression of Caspase-3 in the skin tissue of mice treated topically and exposed to acute UV-B light varied according to the treatment group. The healthy rat group without UV-B (K1) exposure had an average Caspase-3 expression of 0,83±0,11, while the group of mice given a gel base without *Clitoria ternatea* extract and exposed to UV-B (K2) showed slightly higher expression, which was 0,89 ± 0,38. The absence of a significant difference between K1 and K2 suggests that the gel base does not exert a significant influence on Caspase-3 expression, and UV-B exposure under these conditions may not be sufficient to significantly increase apoptosis protein expression (Xi et al., 2016).

These findings are contrary to the general literature, which states that UV-B exposure can increase the expression of apoptotic proteins such as Caspase-3. For example, a study in mouse corneal epithelial cells showed that 2 h of UV-B exposure increased Caspase-3 mRNA expression for up to 6 h, with peak expression at that time. In addition, another study in human skin fibroblasts reported that UV-B exposure at a dose of 150 mJ/cm^2 could activate Caspase-3 and -8, which play a role in the intrinsic and extrinsic pathways of apoptosis (Du et al., 2022).

The group administered a 5% dose of *Clitoria ternatea* extract gel (K3) showed a drastic decrease in Caspase-3 expression, namely 0,16 ± 0,32. Significant differences between K1 and K3 suggest that a 5% dose *of Clitoria ternatea* extract has a pronounced protective effect by suppressing the expression of Caspase-3, which is a key biomarker in the apoptosis pathway. This indicates that this plant extract may play a role in reducing cell death caused by UV-B exposure. On the other hand, the group that was administered a 10% dose of *Clitoria ternata* extract gel (K4) showed a significant increase in Caspase-3 expression, namely 1,52 ± 0,50. However, there were no significant differences between K1 and K4, K2 and K3, K2 and K4, and K3 and K4, suggesting that the protective effect of the 10% dose was inconsistent and likely triggered apoptosis in some cells.

These results showed that a 5% dose of the extract was more effective in lowering Caspase-3 expression, whereas a 10% dose provided less protective effect against apoptosis and even tended to increase Caspase-3 expression. This could indicate the existence of an optimal dose threshold, where dose increases above 5% are not necessarily directly proportional to the protective effect, and may trigger other biological responses that require further research (Cahyani et al., 2023; Saritani et al., 2021).

The results showed that the administration of *Clitoria ternatea extract* gel affected the expression of TNF-α and Caspase-3 in the skin of rats exposed to acute UV-B rays. TNF-α

expression, as an indicator of inflammation, significantly decreased in the group treated with the 5% extract, but a dose increase of up to 10% did not show any significant additional effects. This indicates that 5% is the optimal dose for suppressing the inflammatory response due to UV-B exposure. The expression of Caspase-3, as a marker of apoptosis (Xi et al., 2016) decreased drastically in the group with the 5% extract, showing a protective effect in preventing cell death due to oxidative stress. However, increasing the dose by up to 10% actually triggers higher expression of Caspase-3, indicating the possibility of activation of the apoptosis pathway at higher doses (Jomova et al., 2023).

The results of the analysis showed that the healthy group without UV-B (K1) exposure and the group administered the gel base without the extract showed no significant difference, indicating that the gel base did not significantly affect the expression of Caspase-3. The group treated with a 5% *dose of Clitoria ternatea* extract gel showed a significant decrease in Caspase-3 expression, showing a protective effect in suppressing apoptosis due to UV-B exposure. In contrast, the group with a 10% dose (K4) actually showed increased expression of Caspase-3, indicating that higher doses do not necessarily enhance the protective effect and may trigger other biological responses (Jomova et al., 2023; Na & Ryu, 2018).

This finding is in line with the results of a previous study, which showed that a dose of 5% was more effective in suppressing the inflammatory and apoptotic responses caused by UV-B exposure. In addition, other studies indicate that butterfly pea flower extract has antioxidant properties that can protect the skin from damage caused by UV radiation (Muna et al., 2023). Studies that support these findings include examining the effects of administering butterfly pea flower extract gel on the expression of TNF-α and Caspase-3 in Wistar rats exposed to UVB. The results showed that a dose of 5% was effective in reducing the expression of TNF-α and Caspase-3, while a dose of 10% did not provide any significant additional effects (Cahyani et al., 2023). In addition, another study found that 5% Clitoria ternatea L. extract gel can inhibit the increase in MMP-1 expression in the skin of Wistar rats exposed to UV-B light, supporting the protective effect of this extract against UV-B-induced skin damage. This study examined the effect of applying a butterfly pea flower extract gel as an additional scaling and root planing agent in chronic periodontitis therapy in Wistar rats. The results showed a decrease in TNF-α levels, indicating the anti-inflammatory potential of butterfly pea flower extract (Subchan et al., 2022). Overall, these studies support the finding that Clitoria ternatea extract, particularly at a dose of 5%, has anti-inflammatory and protective effects against UV-B-induced cell damage (Al-Snafi, 2016; Jeyaraj et al., 2022; Subchan et al., 2022).

This study showed the protective effect of 5% C. ternatea extract against inflammation and apoptosis due to UV-B exposure, but had some limitations. This study also tested only two doses, 5% and 10%, so the optimal dose range and potential toxicity could not be determined specifically. Another limitation is the possibility that the active substances in the extract cannot be optimally absorbed below the surface of the skin, which can affect their effectiveness. The presence of infection during treatment can also inhibit the specific action of telang flower extract gel in suppressing inflammation and apoptosis. Therefore, further research with tighter control of infectious factors and absorption of active substances is needed to ensure the effectiveness of this extract as a photoprotective agent (Sharma et al., 2022).

This study had several limitations. The duration and intensity of UV-B exposure were not strong enough to trigger significant inflammatory and apoptotic responses, as seen in group K2, which did not show an increase in TNF-α and Caspase-3 expression. In addition, sampling on day 8 was not ideal because the peak expression of both proteins usually occurs within 24–72 h post-exposure. The base gel, without active ingredients, also acts as a physical barrier, passively reducing UV-B penetration and inflammatory responses. A dose of 5% Clitoria ternatea extract showed an optimal protective effect, whereas a dose of 10% triggered apoptosis through pro-oxidant mechanisms or ER stress. These findings emphasize the need for further studies to determine the optimal dose of the extract such that the protective effect remains maximal without causing unwanted biological responses.

Conclusion

This study concluded that Clitoria ternatea extract gel has an effect on the expression of

TNF-α and Caspase-3 in the skin of Wistar rats exposed to acute UV-B light. Topical administration of 5% Clitoria ternatea extract gel significantly decreased the expression of both biomarkers compared to the control, indicating its potential as an anti-inflammatory and antiapoptotic agent. However, the administration of the gel at a concentration of 10% did not have a similar effect. Practically, the 5% Clitoria ternatea extract gel can be considered as a natural ingredient in topical formulations for skin protection against UV-B damage. Clinical trials in humans, testing of optimal doses, and potential combinations with other protective agents are recommended to support the development of products based on this extract.

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