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Elvana Cahyani
*Biomedical Postgraduate Student, Faculty of Medicine, Sultan Agung Islamic University, Semarang 50112, Indonesia*, elvanacahyani@gmail.com

Agung Putra
*Stem Cell and Cancer Research Indonesia, Semarang 50222, Indonesia*, dr.agungptr@gmail.com

Prasetyowati Subchan
*Biomedical Postgraduate Student, Faculty of Medicine, Sultan Agung Islamic University, Semarang 50112, Indonesia*, psubchan@gmail.com



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# Potential Use of the Gel Extract of Butterfly Pea Flower as Topical Therapy to Prevent Photodamage by Downregulating TNF-α and Caspase-3 Expression Levels in UVB-Exposed Rats

Elvana Cahyani[1*], Agung Putra[1,2], Prasetyowati Subchan[1]

[1]Biomedical Postgraduate Student, Faculty of Medicine, Sultan Agung Islamic University, Semarang 50112, Indonesia
[2]Stem Cell and Cancer Research Indonesia, Semarang 50222, Indonesia

**Abstract**

**Background**: Prolonged exposure to UVB radiation causes DNA damage in skin cells by raising the levels of reactive oxygen species, resulting in the production of inflammatory factors and skin issues. Plant extracts are frequently used to counteract photodamage due to their antioxidant properties. One example is the floral extract of the butterfly pea plant, which contains flavonoid antioxidants. However, the effect of the extract on inflammatory factors is unknown. This study investigated how tumor necrosis factor-alpha (TNF-α) and caspase-3 expression changed when a butterfly pea flower extract gel was applied topically to UVB-exposed animals.

**Methods**: Experimental and control groups were tested. The healthy group was not exposed to UVB. The negative controls and treatments 1 and 2 were exposed daily for 5 days at a minimal erythema dose of 160 mJ/cm$^2$ and then treated with a gel-based extract containing 5% and 10% of the extract, respectively. A 96% ethanol solution was used during the maceration step for the extraction. Real-Time Quantitative Reverse Transcription PCR was used to examine gene expression levels in the skin tissue on day 14.

**Results**: The expression levels of TNF-α and caspase-3 decreased in the treatment group, and higher doses of the extract had a greater effect.

**Conclusions**: The gel extract considerably reduced the UVB-induced TNF-α and caspase-3 production in rats.

**Keywords:** butterfly pea flower, caspase-3, TNF-α, UVB

## INTRODUCTION

UVB radiation is the UV light that penetrates the epidermis and reaches the upper part of the dermis where it induces DNA damage to the skin cells by increasing the concentration of reactive oxygen species (ROS).[1] Excessive ROS production increases inflammation characterized by the release of various proinflammatory molecules, such as tumor necrosis factor-alpha (TNF-α).[2] UV irradiation changes dermal collagen through the collagen breakdown pathway (matrix metalloproteinase proteins [MMPs]), and by inhibiting the procollagen synthesis pathway resulting in the loss of collagen content.[3] UV-induced ROS damage DNA, and induce lipid peroxidation and protein degradation in skin cells. Additionally, ROS reduce the activities of antioxidant enzymes in the skin, including superoxide dismutase and glutathione peroxidase.[4]

Prolonged production of TNF-α induces ROS that cause oxidative damage to DNA through the NADPH-oxidase 1 pathway,[5] thereby activating the p53 gene, which leads to caspase-3 expression and triggers apoptosis in skin cells, including fibroblasts.[6,7] Several studies have revealed that chronic UVB exposure causes oxidative stress, thereby activating the phosphorylation of mitogen-activated protein kinases, as well as the p38, JNK, ERK, and p53 pathways, which trigger the expression of MMPs and degradation of the extracellular matrix, such as collagen.[8,9] Treatments with various chemical agents, such as retinoic, kojic, glycolic acid, hydroquinone, or alpha arbutin, are the main choices but these chemical agents can cause side effects, such as skin irritation, contact dermatitis, genotoxicity, and skin cancer.[10]

All parts of the butterfly pea flower (*Clitorea ternatea*. L) plant,[11] including the roots, seeds, and leaves have been used medicinally and are recognized to have various inflammation-reducing effects.[12] The petals of the butterfly pea flower are a source of anthocyanins and various types of flavonoids with antioxidant effects. Recent studies have reported that butterfly pea extract has high antioxidant activities that inhibit the production of ROS and reduce inflammation, which inhibits the increase in MMP,[12,13] prevents fibroblast cell apoptosis, and inhibits the decrease in collagen.[14] Another study

***Corresponding author:**
Elvana Cahyani
Biomedical Postgraduate Student, Faculty of Medicine,
Sultan Agung Islamic University, Semarang, Indonesia
E-mail: elvanacahyani@gmail.com

demonstrated that anthocyanins are strong antioxidants that reduce ROS if administered topically or orally.[15] However, no study has examined the role of butterfly pea extract on the expression levels of TNF-α and caspase-3 in the skin with low collagen levels due to UVB exposure.[15] Thus, this study examined the effect of topically applied butterfly pea flower extract gel on the expression levels of TNF-α and caspase-3 in Wistar rats exposed to UVB.

## METHODS

### Materials and instruments

The following materials were used in this study: butterfly pea extract, water-based gel, rat caspase-3 primer set (F: 5'-GTGGAACTGACGATGATATGGC-3'; R: 5'-CGCAAAGTGACTGGATGAACC-3' and TNF-α F: 5'-AAATGGGCTCCCTCTCATCAGTTC-3'; R: 5'-TCTGCTTGGTGGTTTGCTACGAC-3'), RNAlater solution, and neutral-buffered formalin. The instruments used were a microscope (Olympus, Tokyo, Japan), UVB tool (25 watts), micropipettes, glass objects and tools, refrigerator (4 °C), freezer (−20 °C and −80 °C), probe sonicator, vortex, and Falcon tubes.

### Preparation of the butterfly pea flower extract

The butterfly pea flowers were purchased from Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional Tawangmangu, Central Java, Indonesia, and extracted at the Integrated Laboratory of Diponegoro University. The extraction was completed using the maceration method with methanol. The filtrate was evaporated to dryness to obtain the flower extract.

### Qualitative phytochemical analysis

The crude extract was screened to detect the secondary metabolites. Alkaloids were detected using the Wagner method, flavonoids using the Willstatter method, tannins using 1% $FeCl_3$, and triterpenoids using the Lieberman–Burchard method.

### UVB-exposed rats

All animal experiments were approved by the Bioethics Commission for Medical/Health Research, Faculty of Medicine, Sultan Agung Islamic University, Semarang No. 304/VIII/2022/Komisi Bioetik. Twenty-four male Wistar rats (*Rattus novergicus*; age, 3 months old; weight, 200 g) were procured from the Faculty of Medicine, Islamic University of Sultan Agung. The rats were quarantined for 3 days and evaluated before the study began. All rats received a standard diet and water *ad libitum* and were divided into four groups consisting of six rats each. The first, second, and third groups included rats having collagen exposed to UVB. The fourth group was not exposed to UV radiation. The hair on the left dorsum of the rats was shaved to maximize the effect of UVB exposure on the skin. UVB was provided five times at an intensity of 160 mJ/cm$^2$/day, for a total dose of 800 mJ/cm$^2$.

### Preparation of the topical gel therapy

The skin surface was treated topically to modify the epidermis and dermis. Topical therapy doses of 5% and 10% were prepared in a water-based gel. The topical gel extract was administered to the rats once a day for 2 weeks after UVB exposure on day 6. Total RNA was extracted from a 50 mg skin tissue sample using the FAVORGEN RNA isolation kit. cDNA was prepared from 25 µg of total RNA in the skin samples using the ReverTra Ace™ qPCR RT Master Mix.

Polymerase chain reaction analysis was conducted to determine the gene expression levels of TNF-α, caspase-3, and the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase using rat-specific primers and SYBR Green DNA polymerase. The reaction was run for 40 cycles at 60 °C for the annealing process.

### Statistical analysis

Data analysis was performed using SPSS software (version 20.0; SPSS Inc., Chicago, IL, USA). Comparative analyses were performed using one-way analysis of variance followed by a post-hoc test to detect differences between the groups. A p-value <0.05 was considered significant.

## RESULTS

### Bioactive compounds in the butterfly pea flower extract

The qualitative test was a phytochemical screening method carried out using colorimetry. The secondary metabolites (phytochemical compounds) were assessed by visualizing the colors produced by each compound. The phytochemical screening included tests such as, flavonoid, alkaloid, saponin, tannin, steroid, and triterpenoid tests.

This type of phytochemical screening analysis provides valuable information regarding the presence of the important classes of phytochemicals in an extract. The results indicated that flavonoids could be a potential source of antioxidant activity in butterfly pea flower extract. A quantitative flavonoid test was performed to analyze the total flavonoid contents in the ethyl acetate and ethanol extracts of butterfly pea flowers. The total flavonoid levels were measured three times and the results are shown in Table 1. The average total flavonoid content in a butterfly pea flower extract sample was 682,0238.

**TABLE 1.** Total flavonoids in the butterfly pea flower extract

| Extract Concentration | Total Flavonoid Compound |
|---|---|
| 1000 ppm | 690,2143 |
| 1000 ppm | 671,6429 |
| 1000 ppm | 684,2143 |

**Butterfly pea flower extract gel increases collagen density in the skin of UVB-exposed rats**

*Collagen density profile in UVB-exposed rats*

The rats were stimulated with UVB light for 5 days at an intensity of 160 mJ every 15 min and then euthanized under anesthesia. Mason's trichrome staining was used to examine UVB-exposed rat skin tissue. The UVB-exposed rats expressed less collagen than the healthy rats. As shown in Figure 1, the healthy rat skin displayed greater collagen density (blue hue) than the rat skin exposed to UVB radiation.

*Expression levels of TNF-α and caspase-3 in dermal fibroblasts*

The effect of the butterfly pea flower extract topical gel on relative TNF-α mRNA expression level was analyzed using Real-Time Quantitative Reverse Transcription PCR. The expression levels of TNF-α and caspase-3 mRNA decreased significantly in the dorsal skin of the treatment group than in the control group. However, the expression levels of TNF-α and caspase-3 mRNA were higher in the UVB group than in the treatment group.

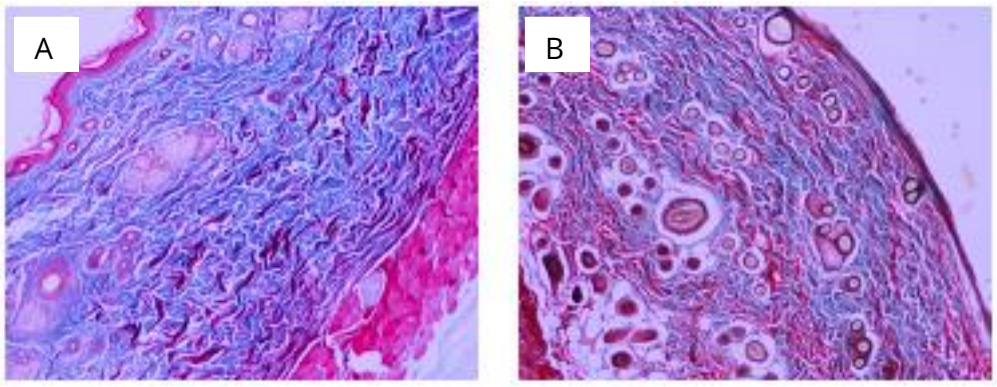
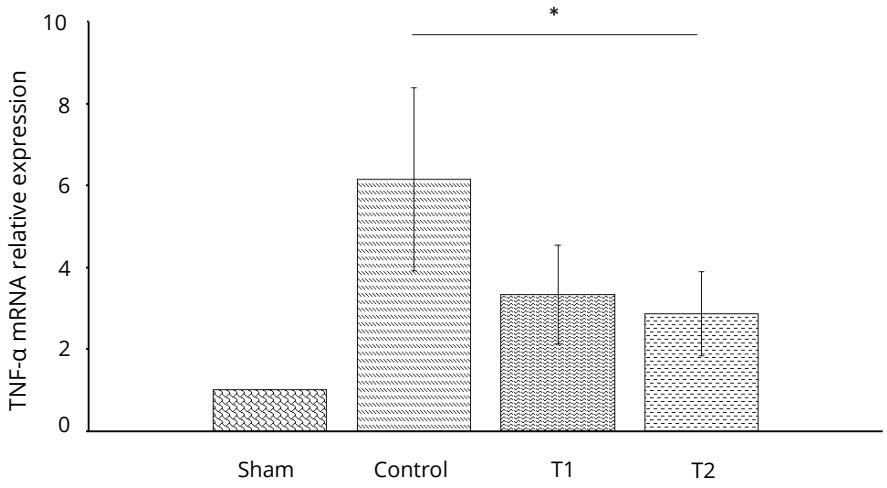
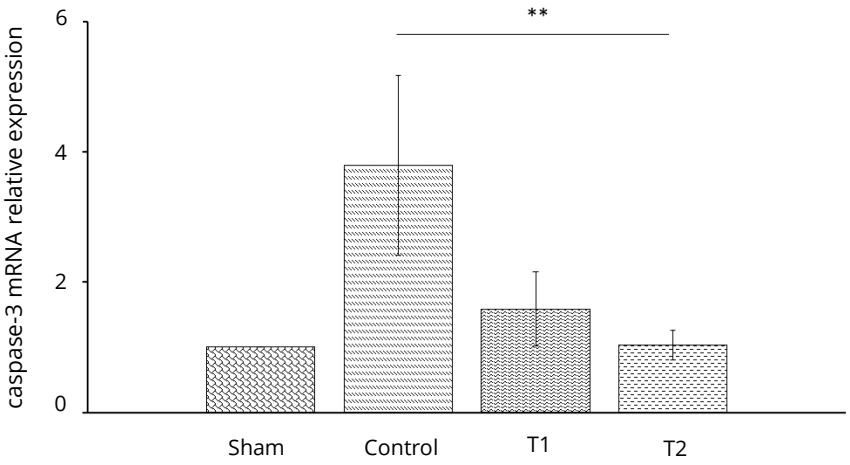



**FIGURE 1.** (A) Healthy rat collagen (B) Reduced collagen density caused by UVB exposure of rat skin tissue.




*significant difference in the control group ($p < 0.05$); indicates a significant difference in the UVB group (N = 6 per group).

**FIGURE 2.** Relative expression levels of TNF-α in the dorsal skin observed using real-time polymerase chain reaction: **(Sham)** group not exposed to UV radiation, **(Control)** group with UV-exposed collagen loss, **(T1)** group with collagen exposed to UVB-treated topical gel from 5% of butterfly pea extract, **(T2)** and group with collagen exposed to UVB-treated topical gel from 10% extract of butterfly pea.




*significant difference in the control group ($p < 0.01$); indicates a significant difference in the UVB group (N = 6 per group).

**FIGURE 3.** Relative expression levels of caspase-3 in the dorsal skin observed using real-time polymerase chain reaction. **(Sham)** group not exposed to UV radiation, **(Control)** group with UV-exposed collagen loss, **(T1)** group with collagen exposed to UVB-treated topical gel from 5% of butterfly pea extract, **(T2)** and group with collagen loss exposed to UVB-treated topical gel from 10% of butterfly pea extract.

The results of the TNF-α gene expression level analysis on day 14 after UVB exposure are shown in Figure 2. The results show that the gel topical therapy based on the butterfly pea flower extract decreased the expression of TNF-α in rat UVB-exposed skin. TNF-α gene expression level in T2 was significantly different from that of the control and healthy groups ($p < 0.05$) but was not significant compared with T1 ($p > 0.05$). The data also shows that T1 was not significantly different from the control ($p > 0.05$) but was significantly different from the healthy rats ($p < 0.05$).

The results of the caspase-3 gene expression level analysis on day 14 after UVB exposure are shown in Figure 3. The results show that the topical gel therapy based on the butterfly pea flower extract decreased the expression of caspase-3 in rat UVB-exposed skin. Caspase-3 gene expression was significantly different in T2 from that of the control and healthy groups ($p < 0.05$) but was not significant compared with T1 ($p > 0.05$; Mann–Whitney). The data also shows that T1 was not significantly different from the control ($p > 0.05$) but was significantly different from the healthy rats ($p < 0.05$).

## DISCUSSION

UV radiation increases ROS production and activates signal transduction pathways leading to tissue damage. In addition, ROS produced by UVB irradiation increase the levels of inflammatory factors, such as TNF-α, which modulate cell apoptosis by activating caspase-3, causing a decrease in collagen and other skin problems.[17,18]

The butterfly pea flower has been proposed to have potent antioxidant activities. The antioxidant capacity of a coffee extract is determined by its high flavonoid contents, such as anthocyanins, quercetin alkaloids, saponins, and tannins. Anthocyanins are a class of flavonoids that are potential photoprotective agents because they absorb UV rays 31.99 and act as antioxidants and anti-inflammatory compounds.[12,19]

In the present study, applying 10% butterfly pea flower extract gel topically inhibited photodamage by downregulating the expression levels of the TNF-α and caspase-3 genes and significantly reducing the TNF-α levels ($p < 0.05$). The flavonoids in the butterfly pea flower extract may have caused the decrease in the TNF-α level. A previous study reported the role of anthocyanins in the activation of Nrf-2, which directly inactivates NF-kB.[20]

Recent studies have suggested that NF-B plays a crucial role in the development of skin inflammation due to UVB exposure. Inhibiting NF-kB expression may suppress skin inflammation in response to UVB radiation. Based on previous studies, activating NF-kB triggers the release of inflammatory cytokines, such as interferon-γ, interleukin (IL)-6, and TNF-α.[21,22] The NF-kB signaling pathway is activated in response to different stimuli, including the cytokine TNF-α, which is secreted by local macrophages during infection and cellular stress caused by external factors, such as exposure to UV light. In the absence of activating stimuli, NF-kB dimers are retained in the cytoplasm in association with inhibitory members of the NF-kB inhibitory (IkB) protein family.[23,24]

UVB exposure releases the NF-KB bonds and inhibiting factors so that NF-kB is activated. Previous studies have reported the role of a butterfly pea flower extract in inhibiting the expression of NF-kB. Suppressing the

transcription factor NF-kB is associated with the cleavage of the inflammatory cytokine pathway, including TNF-α. This aligns with the results of this study, which found a decrease in TNF-α expression after the administration of butterfly pea flower extract gel.[25–27]

TNF-α signals the regulation of immune homeostasis and is involved in the regulation of cell death.[28] The TNF-α-associated apoptotic mechanism is closely related to a cascade of apoptotic cysteine proteases known as caspases, which are responsible for initiating and executing apoptosis. The death signal from the TNF-α receptor is transduced to the TNFRSF1A Associated Via Death Domain adapter protein, which uses the subsequent Fas Associated Via Death Domain adapter protein and regulates The death-inducing signaling complex to activate caspase-8 to caspase-3, leading to cell apoptosis. The decreased expression of TNF-α is correlated with the blocked production signal of the apoptotic enzymes, including caspase-3.[29]

Furthermore, a significant decrease in caspase-3 expression level was detected in the T2 group compared with the control group. This result may be due to the decrease in TNF-α expression level in group 2. This result aligns with the previous studies, stating that a decrease in caspase-3 and TNF-α expression levels in the skin tissue of UVB-exposed rats after administering butterfly pea flower extract gel has implications for stopping the death of the skin cells.

Mason's trichrome staining indicated that UVB exposure decreased collagen density in the test group of rats, indicated by a reduced blue hue on the stained slides. This finding demonstrates that an inflammatory response of the skin was successfully induced by UVB after 5 days of treatment at a level of 160 Mj/cm$^2$ for 15 min each day. UVB irradiation induced ROS production in the epidermis, which caused the release of IL-6, and overproduction of MMPs activated by the protein transcription factor AP-1. MMPs destroy collagen and decrease collagen density.

Based on the results of this study, administering butterfly pea flower extract maintained the firmness of the skin exposed to UVB by inhibiting TNF-α and caspase-3 expression levels; however, this study had several limitations, such as the collagen level was not verified in the skin.

## CONCLUSIONS

The butterfly pea flower extract gel was useful as a topical treatment for photodamage caused by excessive decreases in caspase 3 and TNF-α gene expression levels on the UVB-exposed skin of rats. This study will serve as the basis for further applied research, leading to the production of photoaging products, such as hyperpigmentation therapy.

## CONFLICTS OF INTEREST

None declared.

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