The Influence of Blue Butterfly Pea Flower (Clitoria Ternatea) Gel Extract on Interleukin-10 (IL-10) and Glutathione Peroxidase (GPx) Levels

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ABSTRACT

Long-term exposure to Ultraviolet B (UV-B) radiation causes increased skin darkening owing to a reaction to oxidative stress leading to elevated levels of nitric oxide (NO) and reactive oxygen species (ROS). Excessive ROS induces signal transduction and stimulates the transcription factor NF-κB, an inflammatory mediator. Butterfly flower extract has high antioxidant levels, inhibiting ROS production and reducing inflammatory conditions, hindering MMP, preventing fibroblast cell apoptosis, and inhibiting collagen degradation. However, the role of butterfly pea flowers on IL-10 and GPx gene levels in melasma skin due to UV-B exposure is unclear. This research aims to assess the effectiveness of applying Blue Butterfly Pea Flower Extract Gel on the level of the IL-10 and GPx genes in Wistar rat strains exposed to UV-B. The UV-B-experimental research with the post-test control group. Groups K2, K3, and K4 were each exposed to UV-B at 302 nm with a MED of 160 mJ/cm², while group K1 was the healthy group. K3 was given 5% butterfly pea flower gel, K4 was given 10% gel daily for 14 days, and K2 received base gel. On the 21st day, ELISA examined the tissue for IL-10 and GPx levels. The IL-10 gene level in the treatment group increased with higher dosages (K3=83.27±3.11, K4=90.66±4.00) compared to controls (K2=33.26±2.98, K1=104.7±3.26). The relative level of the GPx gene in the treatment group increased along with increasing dose (K3=44.90±1.44, K4=54.09±1.00) compared to the control group (K2=29.54±0.85, K1=62.43±0.85). Administration of butterfly pea flower gel can increase the level of the IL-10 gene and the level of the GPx gene in the skin tissue of mouse models of UV-B light-induced hyperpigmentation.

Keywords: Gel; Butterfly Flower; IL-10; GPx; Hyperpigmentation
INTRODUCTION

Sustained exposure to Ultraviolet B (UV-B) radiation induces hyperpigmentation via oxidative stress response, thereby triggering an increase in nitric oxide (NO) and reactive oxygen species (ROS) levels. (1,2) Excess reactive oxygen species (ROS) induce signal transduction and activate the nuclear factor kappa β (NF-κB) transcription factor, which acts as an inflammatory mediator. (3) The interaction between the heterodimer subunits P50 and p65 NF-κB triggers an inflammatory response that leads to the secretion of proinflammatory cytokines such as interleukin-6 (IL-6), interleukin-1β (IL-1β), and interleukin-10 (IL-10), causing inflammation in the skin. (4) UV-induced ROS causes DNA damage, lipid peroxidation, and protein degradation in skin cells. These also reduce the activity of antioxidant enzymes in the skin, such as glutathione peroxidase (GPx). (5) Low serum glutathione levels disrupt the regulation of immune cell functions by reducing calcium mobilization and tyrosine phosphorylation in proteins, including forming the CD3 immune cell receptor. The GCS gene synthesizes glutathione, and alterations in the levels of this gene impair GSH synthesis, potentially worsening disease severity. (6) The gel extract from Clitoria ternatea L. inhibits the increase of MMP-1 levels in the skin of Wistar rats exposed to UV-B radiation. (7)

The prevalence of melasma globally, especially in Brazil, reaches 5.3% to 9.1%. A study in India found that UV exposure risks nearly half of melasma patients, around 48.8%, with 58.1% working outdoors, increasing their risk of high UV exposure. (8) Dr. Soetomo Hospital in Surabaya recorded a melasma incidence of 5% from 2012 to 2014. From 2016 to 2018, 60 new melasma patients were identified, constituting 0.10% of the total 560 patients in the dermatology and venereology outpatient clinic. (9) Long-term exposure to UV-B radiation causes oxidative stress, which activates specific proteins called mitogen-activated protein kinases (MAPK), such as p38, JNK, ERK, and p53. This activation produces enzymes called matrix metalloproteinases (MMPs), leading to the breakdown of the extracellular matrix (ECM). (10,11) UV exposure increases melanocyte proliferation and activity, causing epidermal pigmentation in sun-exposed areas. Repeated suberythemal doses stimulate melanogenesis, increasing melanin in the epidermis and dermis, as seen in melasma's microscopic findings. UV radiation activates protein kinase C (PKC) through diacylglycerol (DAG), triggering melanogenesis. The relationship between this process and DNA damage or the DAG pathway involving arachidonic acid in melasma remains unclear. (12,13) The butterfly pea flower, rich in nutrients, has long been recognized as a medicinal plant. People use all parts of the plant for medicinal purposes, mainly to reduce inflammation. (14,15)

The petals of the butterfly pea flower contain anthocyanins and flavonoids with antioxidant effects. (16,17) Cahyaningsih et al. demonstrated that butterfly pea flower extract has high antioxidant properties, inhibiting ROS and reducing inflammation. This inhibition can prevent MMP activity, fibroblast cell apoptosis, and collagen degradation. Anthocyanin, the main component, is a powerful antioxidant used topically or orally. Secondary metabolites in the ethanol extract of C. ternatea, such as
flavonoids, saponins, terpenoids, and tannins, also contribute to antioxidant activity, with an IC50 value of 87.86 ppm, indicating strong antioxidant potency. \(^{(18)}\) Rahayu et al. found antioxidant activity in butterfly pea flowers from North Lombok and Wonosobo with IC50 values of 4.19 ppm and 3.08 ppm, respectively. \(^{(19)}\) Zakaria et al. proved the protective effect of C. ternatea flower extract against skin aging, suggesting its use in cosmetics and traditional medicine. \(^{(20)}\) Zagórska-Dziok et al. demonstrated that butterfly pea flowers have anti-inflammatory and anti-aging potential as they can inhibit collagenase enzymes. \(^{(21)}\) Jayanti et al. found that lotion containing butterfly pea flower extract exhibits strong antioxidant properties and can protect the skin from free radicals. \(^{(22)}\) Suherlan et al. reported ternatin A1, a compound found in butterfly pea flowers, as a promising candidate for antimelanogenesis. They did not recommend ternatin D1, ternatin B1, and ternatin C1 as candidates for antimelanogenesis. \(^{(23)}\) Currently, no studies exist on the role of butterfly pea flowers in IL-10 and GPx gene levels in UV-B-induced melasma-affected skin. This research will evaluate the effects of butterfly pea flower gel extract on IL-10 and GPx gene levels in UV-B-exposed Wistar rats using macroscopic and microscopic examinations.

**METHOD**

This study is an experimental investigation conducted on male Wistar rats using a post-test-only control group design. Figure 1 randomly divides the research subjects into four groups: K1 as the control without UV exposure, K2 with UV-B exposure, K3 with 5% butterfly pea flower gel extract and UV-B exposure, and K4 with 10% butterfly pea flower gel extract and UV-B exposure. Observations are conducted on each group (OK1 for K1, OK2 for K2, OK3 for K3, and OK4 for K4).

![Figure 1. Post-test control group design](image)

**Research Population**

The research population consists of male Wistar rats, aged 10-12 weeks, weighing 180-220 grams, maintained at the PSPG UGM Animal Laboratory. They are fed a regular Citrafeed diet and provided with equal amounts of water. The rats are acclimatized for seven days before the treatment. Simple random sampling procedures are used to select 24 rats, which are then divided into four groups: one control group and three treatment groups. To meet inclusion criteria, rats must be healthy and free...
from morphological abnormalities. Any presence of anatomical abnormalities or previous participation in studies serves as grounds for exclusion. Sample size is determined according to WHO guidelines, with a minimum of 5 rats per group and an additional 10% as reserves. In this study, the dose of Butterfly Pea Flower Extract Gel is the independent variable, while the levels of IL-10 and GPx are the dependent variables.

Research Tools and Materials

This study employs various equipment, including a UV lamp with an energy of 160 mJ/cm², electric hair clippers, exposure cages, maintenance cages, and rat drinking water dispensers. For data collection, swing bucket centrifugation, EDTA vacutainers, hematocrit tubes, 5 mL pots, 6 mm biopsy punches, micropipettes, 1000 uL micropipette tips, and 1.5 mL vial tubes are utilized. Data analysis is conducted using RT-PCR. Materials utilized comprise butterfly pea flower extract, RNA later, PBS (phosphate-buffered saline), DNA isolation kit, PCR analysis kit, aquades water, ketamine, xylazine-based water gel, ethanol, distilled water, rat food, and chloroform.

Figure 2. Research Design

Figure 2 illustrates that 24 healthy male Wistar rats were prepared for seven days before UV-B radiation exposure with a dose of 160 mJ/cm² for five days. Subsequently, the rats were randomly allocated into four groups containing six samples. Group I consisted of healthy rats without prior...
exposure. Group II was the positive control group, where the rats were exposed to UV-B and topically treated with base gel. Group III was the first treatment group, where the rats were subjected to UV-B exposure and topically treated with 5% butterfly pea flower extract gel. Group IV was the second treatment group, where the rats were exposed to UV-B radiation and topically treated with 10% butterfly pea flower extract gel.

In this research, skin tissue samples were taken on Day 20 to analyze the levels of the IL-10 gene and GPx enzyme. Rat blood samples were collected from the orbital sinus using microhematocrit capillaries and separated into serum and blood cells by centrifugation. Reagents, samples, and standard solutions were prepared after being at room temperature for 30 minutes. Healthy plates and strips were ready, with unused strips stored at 2-8°C. Standard solutions were added to the wells, followed by samples and anti-GPx antibodies. Then, streptavidin-HRP was added except for the negative control, and incubation was carried out at 37°C for 1 hour. Wells were washed and incubated with substrate solutions A and B for 10 minutes until the solution changed color. Finally, absorbance was read at a wavelength of 450 nm using an ELISA reader, with valid readings performed within less than 10 minutes.

The findings were evaluated to gather essential data through data processing, editing, and tabulation before conducting descriptive tests. Shapiro-Wilk test was used for normality testing, while Levene's test was used for variance testing. One-way ANOVA and Post Hoc tests were performed when data distribution was normal and variances were equal. Kruskal-Wallis and Mann-Whitney tests were used for non-normal data distribution and unequal variances. This study was conducted using SPSS software on the Windows operating system.

RESULT

The experiment induced hyperpigmentation using UV-B 302 nm at an intensity of 160 mJ/cm2 on 24 male Wistar rats. Researchers divided the rats into four groups: two treatment groups (K1 and K2) with six rats each, treated with 5% and 10% butterfly pea flower gel respectively; one positive UV-B group that received UV-B exposure with base gel treatment; and one healthy group without treatment. They adapted the rats for seven days, treated them with UV-B radiation for five days, and then gave them 5% and 10% butterfly pea flower gel in K1 and K2 for 14 days. Researchers took samples on day 21 for data analysis.

Table 1. Results of Mean and Standard Deviation of Il-10 Gene Expression and GPx Gene Expression

<table>
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<th>Variabel</th>
<th>Ekspresi Gen IL-10</th>
<th>Ekspresi Gen GPx</th>
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<tr>
<td>K1</td>
<td>104,7±3,26</td>
<td>62,43±0,85</td>
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<tr>
<td>K2</td>
<td>33,16±2,98</td>
<td>29,54±0,85</td>
</tr>
<tr>
<td>K3</td>
<td>83,27±3,11</td>
<td>44,90±1,44</td>
</tr>
<tr>
<td>K4</td>
<td>90,66±4,00</td>
<td>54,09±1,00</td>
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Table 2. Results of Shapiro Wilk IL-10 Gene Expression and GPx Gene Expression

<table>
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<th>Ekspresi Gen GPx</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0,807</td>
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</tr>
<tr>
<td>K4</td>
<td>0,677</td>
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</table>

Figure 3. Results of Levene's Test for IL-10 Gene Expression and GPx Gene Expression

Table 1 shows the highest average IL-10 gene levels in group K1, followed sequentially by K4, K3, and K2. The IL-10 gene levels from the four groups display a normal distribution based on the Shapiro-Wilk test (p > 0.05, Table 2). According to the Levene's test, they have uniform data variance (p > 0.05, Figure 3). Parametric statistical analysis was conducted using the One-Way ANOVA test on data with normal distribution and homogeneous variance. The test yielded a p-value of 0.000 (p<0.05), indicating a significant difference in the average IL-10 gene levels among the four groups. Following significant results in the One-Way ANOVA test, a Post Hoc test was performed to identify the butterfly pea flower gel dose with the most significant effect.

The GPx gene has the highest average levels in group K1, followed sequentially by K4, K3, and K2, as shown in Table 1. The GPx gene levels from the four groups display a normal distribution based on the Shapiro-Wilk test (Table 2) with a p-value greater than 0.05. Additionally, according to the Levene's test (Figure 3), the data variance is homogeneous, with a p-value greater than 0.05. Parametric statistical analysis was conducted using the One-Way ANOVA test on data with normal distribution and homogeneous variance. The test yielded a p-value of 0.000 (p<0.05), indicating a significant difference in the average GPx gene levels among the four groups. Following significant results in the One-Way ANOVA test, a Post Hoc test was performed to identify the butterfly pea flower gel dose with the most significant impact.
IL-10 Gene Level

The Post Hoc test showed a p-value of < 0.05 for comparing the mean IL-10 levels between K1 and K2, K3, and K4 (0.000), indicating a significant difference. There was a significant difference between the means of K1 and K2 (0.000), K2 and K3 (0.000), and K3 and K4 (0.001). The results of the LSD Post Hoc test indicated that both doses of butterfly pea flower gel, 5% and 10%, could increase IL-10 gene levels in male Wistar rats with hyperpigmentation. Although both doses could enhance IL-10 gene levels, the treatment in K4 had a more significant effect than the treatment in K3.

![Figure 4. IL-10 Gene Level](image)

Exposure to moderate to high levels of UV-B radiation can cause oxidative stress, leading to skin hyperpigmentation. Melanocyte cells produce cytokines like IL-10, which inhibit the accumulation of reactive oxygen species (ROS) as seen in Figure 4.

GPx Gene Level

The Post Hoc test showed a p-value < 0.05 for comparing the mean levels of the GPx gene between K1 and K2, K3, and K4 (0.000), indicating a significant difference. There was a significant difference between the means of K1 and K2 (0.000), K1 and K3 (0.000), and between K3 and K4 (0.000). The Post Hoc LSD test results showed that both doses of butterfly pea flower gel, 5% and 10%, could increase the levels of the GPx gene in male Wistar rats with hyperpigmentation. Although both doses could increase GPx gene levels, the K4 treatment had a more significant effect than the K3 treatment.

Hyperpigmentation is a skin issue requiring a tissue regeneration response to inhibit melanogenesis and rebuild collagen. UV-B radiation exposure has been shown to cause collagen damage by increasing the activity of MMP enzymes. Cytokines, including IL-1, IL-6, and TGF-β, may inhibit MMP enzyme production and halt collagen damage by reducing the mitogen-activated protein kinase (MAPK) pathway, thereby increasing GPx gene activity (Figure 5).
DISCUSSION

UV-B radiation exposure increases the levels of proteins that produce melanin, such as MITF, which contributes to skin hyperpigmentation. (24) UV-B radiation damages DNA through oxidative ROS, triggering the melanogenesis pathway. (25) The extract of butterfly pea flower contains various compounds such as flavonoids, phenols, tannins, saponins, phlobatannins, triterpenoids, carbohydrates, alkaloids, glycosides, anthraquinones, steroids, and volatile oils. (26)

Previous studies have also shown that anthocyanins, especially ternatin A1, A2, B1, B2, D1, D2, Preternatin A3, and A4, as well as delphinidin, are the major flavonoids in butterfly pea flowers. These compounds are believed to play a role in its biological activities. Butterfly pea flowers have been proven to possess various pharmacological activities, including antioxidant, anti-inflammatory, analgesic, antihistaminic, anticancer, antibacterial, and immunomodulatory properties. (27) Flavonoid and phenol are anti-inflammatory compounds found in plants. Flavonoids inhibit various enzymes such as Ca2+-ATPase, xanthine oxidase, phosphodiesterase, aldose reductase, lipoxygenase, and cyclooxygenase. (28) Phenol acts as an anti-inflammatory by inhibiting enzymes cyclooxygenase and prostaglandin, and by neutralizing free radicals that trigger inflammation. (29)

Exposure to UV radiation generates ROS, activating AP-1, leading to decreased levels of genes such as TGF-β1 and IL-10. This results in collagen degradation and increased skin damage, such as sunburn and wrinkles. This study investigates the effects of butterfly pea flower extract gel on hyperpigmented rats and finds that the gel increases IL-10 gene levels, inhibiting hyperpigmentation. Previous research also indicates that butterfly pea flower extract has anti-inflammatory effects by inhibiting NF-κB, directing M1 macrophages toward the M2 phenotype, and increasing levels of VEGF, IL-10, and TGF-β.

Research indicates that butterfly pea flower extract gel inhibits GPx gene levels. The antioxidant compounds in butterfly pea flowers can neutralize free radicals, as evidenced by their ability to form semiquinone radicals and stabilize quinone structures. (25) Previous research confirms that flavonoids, acting as antioxidants, reduce ROS levels and maintain balance by enhancing antioxidant production.
The balance between ROS and antioxidants plays a critical role in several cellular signaling pathways, influencing processes such as differentiation, proliferation, migration, survival, and death.

The enzyme GPx facilitates the transformation of 2 molecules of oxidized GSH into GSSG and two molecules of water (2H₂O). Oxidized GSH, the byproduct of oxygen and lipid metabolism, can cause cell damage if left unregulated. Therefore, GPx is crucial as an antioxidant defense in all cells exposed to oxygen radicals.

GPx plays a role as a factor in inhibiting collagen degradation due to UV-B radiation exposure. UV-B radiation exposure can suppress GPx and cause chronic photo-oxidative stress. Butterfly pea flower extract increases GPx gene levels, especially flavonoids like quercetin, which inhibit the activity of protein kinase kinase (MEK1) and phosphoinositide 3-kinase (PI3K), and activate mitogen-activated protein kinase (MAPK) to enhance antioxidant enzyme activity, thereby reducing oxidative stress by increasing GPx.

**CONCLUSION AND RECOMMENDATIONS**

Exposure to UV-B radiation can increase skin hyperpigmentation through the melanogenesis pathway triggered by DNA damage via oxidative ROS. However, butterfly pea flower extract contains various compounds, especially flavonoids and phenols, with antioxidant and anti-inflammatory activities. Research shows that butterfly pea flower extract gel can inhibit skin hyperpigmentation by increasing IL-10 gene levels and enhancing GPx levels to counter UV-B-induced photo-oxidative stress. Therefore, butterfly pea flower has the potential as an agent to protect the skin from UV-B-induced damage and reduce skin hyperpigmentation processes. Future researchers are advised to complement the analysis by examining ROS, TGF-β, and TRP1/2 levels after administering butterfly pea flower extract gel treatment to understand the molecular mechanisms in preventing hyperpigmentation.

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