Administration of Kakadu Plum Extract (Terminalia ferdinandiana) Cream Prevented the Increase of Tyrosinase Expression and Melanin Amount on Ultraviolet-B Exposed Male Guinea Pigs (Cavia porcellus) Skin

Maulana Setiawan1, Ida Sri Iswari2, Ni Wayan Winarti3

1Master Program in Biomedical Sciences, Anti-Aging Medicine Major, Faculty of Medicine Udayana University, Denpasar, Bali, Indonesia
2Clinical Microbiology Department, Integrated Laboratory Service Unit, Sanglah Hospital, Denpasar, Bali, Indonesia
3Anatomical Pathology Department, Faculty of Medicine Udayana University, Sanglah Hospital, Denpasar, Bali, Indonesia

Abstract: Background: Hyperpigmentation causes various impacts such as lack of confidence and decreased productivity. The gold standard of hyperpigmentation treatment is using 4% hydroquinone that leads to ochronosis, a blue black discoloration in the skin. Thus, it is necessary to find a solution to suppress this side effect. Kakadu plum (Terminalia ferdinandiana) has high ascorbic acid levels, ellagic acid and tanin, which act as an anti-hyperpigmentation agent. This study aims to investigate the effectiveness of Kakadu plum extract cream on male guinea pig’s skin exposed to UV-B, related to its role as an anti-hyperpigmentation agent. Method: This research was an experimental study with a randomized post-test only control group design. This study used 36 healthy male guinea pigs (Cavia porcellus), local strain, three months old, 300-350 grams in weight. The sample was divided into two groups randomly. The control group was given a placebo cream, and the treatment group was given 0.1% Kakadu cream every day, 20 minutes before and 4 hours after UV-B radiation with radiation of 65 mJ/cm2 6 times for two weeks. Tyrosinase expression was measured using the Tyrosinase Examination Kit with the immunohistochemistry staining method. The amount of melanin was calculated using a histopathological method with Masson-Fontana staining. Results: The results showed that the mean tyrosinase expression in the treatment group was significantly lower than the control group (36.19±4.60% vs. 14.99±2.30%; p<0.001). The mean amount of melanin in the treatment group was also significantly lower than the control group (25.93±4.52% vs. 4.40±2.50%; p<0.001). Conclusion: Based on this study's results, it can be concluded that kakadu extract (Terminalia ferdinandiana) cream prevented the increase of tyrosinase expression and melanin amount on ultraviolet B exposed male guinea pigs (Cavia porcellus) skin.

Keywords: melanin, tyrosinase expression, Terminalia ferdinandiana, UVB

1. Introduction

Aging is a natural process that intrinsic and extrinsic factors can influence every living thing experiences. Intrinsic factors include genetic, hormonal, and racial characteristics, while extrinsic factors include ultraviolet (UV) exposure, temperature, humidity, and pollution exposure.1 These risk factors and increasing age will cause photoaging, which generally occurs on the face, neck, chest, and arms because these areas tend to get UV exposure. The characteristics of photoaged skin are skin wrinkled, dry, sagging skin, and pigment disorders such as hyperpigmentation.2

Hyperpigmentation is a body mechanism that aims to prevent skin tissue damage, including the underlying tissue due to exposure to UV rays. The body’s protective mechanism as a protector against UV rays is to form melanin.3 Hyperpigmentation conditions will cause various impacts such as lack of confidence and decreased work productivity. The current gold standard of hyperpigmentation treatment is 4% hydroquinone, but it can cause ochronosis effects. Therefore, it is necessary to develop natural ingredients to suppress the side effects of treatment. Kakadu plum (Terminalia ferdinandiana) is a plant that can hide hyperpigmentation.4

Kakadu plum (Terminalia ferdinandiana) is a plant from Australia. It contains ascorbic acid of 173.5-322.2 mg/g DW, total phenolic of 376.1-1505.2 mg GA E/g DW, and ellagic acid of 3,050-14,020 mg/100 g DW, which has potential effect as anti-hyperpigmentation.5 High antioxidants in Kakadu plum (Terminalia ferdinandiana) will neutralize free radicals due to UV exposure, and Vitamin C can reduce the amount of melanin in the skin through suppression of anti-tyrosinase activity.6 Until now, studies measuring the effectiveness of Kakadu plum (Terminalia ferdinandiana) in vivo as an anti-hyperpigmentation agent on male guinea pig skin have not been conducted. Therefore, this study aims to measure Kakadu plum cream’s effectiveness applied to male guinea pig’s skin exposed to UVB, related to its role as an anti-hyperpigmentation agent.

2. Method

This study was an experimental randomized post-test only control group design, which was conducted for five weeks. Histopathological examination of skin tissue and tyrosinase examination was carried out at the Integrated Biomedical Laboratory Unit, Faculty of Medicine, Universitas Udayana. Meanwhile, phytochemical investigations were carried out at the Department of Agricultural Technology at Universitas Udayana and the
Faculty of Engineering at Universitas Udayana. This study sample was a male guinea pig (Cavia porcellus), three months old, weighing 300-350 grams, given standard dietary animal feed (HI-GRO 552 and vegetables). The food composition of male guinea pigs consists of 3-4% fat, 35-40% carbohydrates, 17-20% protein, and drinking using boiled water ad libitum. Male guinea pigs that die in the study will drop out. The number of samples in each group was 18, with the experimental group being given 0.1% Kakadu cream.

This study's independent variables were 0.1% Kakadu cream and placebo cream; the dependent variables were tyrosinase expression and the amount of melanin. Control variables were age, guinea pig strain, color, genetics, guinea pig feed, guinea pig activity, guinea pig body weight, guinea pig health, and the condition variable is UVB exposure. The Kakadu cream used was 0.1% DBI Kakadu cream produced by PT Derma Beauty Indonesia. In the treatment group, 0.2 mg of Kakadu cream was applied before UVB exposure on the backs of shaved guinea pigs, measuring 2x2cm, for 20 minutes and once a day with each exposure. Male guinea pigs were exposed to UVB rays at a dose of 65 mJ/cm² 3 times a week for 65 seconds, then given Kakadu cream again for 4 hours after exposure. On days without exposure, Kakadu cream is given once a day. In the control group, guinea pigs were given 0.2 mg placebo cream with the same frequency and procedure as the treatment group.

Tyrosinase expression was measured by melanocyte expression in the epidermis using DAKO Envision's anti-tyrosinase primary antibody kit. Tyrosinase enzyme is indicated by the nucleus melanocyte cells that are blue with brown cytoplasm. The tyrosinase expression will be measured by calculating the tyrosinase pixel divided by the epidermal pixel multiplied by 100%. Meanwhile, the amount of melanin will be measured through a tissue biopsy stained with Masson-Fontana. The calculation is done by comparing the amount of melanin seen in pixels with the entire epidermal tissue visible in pixels multiplied by 100%.

All data collected was tested for data normality with Saphiro Wilk and homogeneity test using Levene's test. Furthermore, descriptive analysis and comparative analysis will be carried out using parametric statistical tests with an independent sample t-test because the data is normally distributed (p>0.05).

3. Result

This study divides the subjects into a control group and a treatment group. The control group was given exposure to ultraviolet B ray and placebo cream, while the treatment group was given exposure to ultraviolet B ray and 0.1% Kakadu cream. After being given UV exposure, the treatment group applied with Kakadu cream and showed fewer brown hyperpigmented lesions (Figure 1B). More brownish hyperpigmentation was seen in the control group applied with placebo cream after UV exposure (Figure 1A).

Tyrosinase expression was measured by immunohistochemistry analysis. Tyrosinase expression is shown in Figure 2.
After two weeks of treatment, the guinea pig's back skin tissue was biopsied for histopathological examination. Melanin will be black in the Masson-Fontana stain. The appearance of melanin in the epidermal tissue of guinea pig skin is shown in Figure 3.

![Image](image1.png)

**Figure 3:** Description of Melanin on guinea pig skin with Masson Fontana Staining. Melanin image is shown in black (arrow).

Tyrosinase expression and the amount of melanin were analyzed by descriptive test. This analysis aims to describe the data, including mean, standard deviation (SD), median, minimum, and maximum (Table 1).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>Min.</th>
<th>Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosinase (%)</td>
<td>Placebo Cream 0.1%</td>
<td>36.19</td>
<td>4.60</td>
<td>36.80</td>
<td>28.50</td>
<td>43.80</td>
</tr>
<tr>
<td>Melanin (%)</td>
<td>0.1% Kakadu Cream</td>
<td>14.99</td>
<td>2.30</td>
<td>14.65</td>
<td>10.50</td>
<td>19.30</td>
</tr>
<tr>
<td>Tyrosinase (%)</td>
<td>Placebo Cream 0.1%</td>
<td>25.93</td>
<td>4.52</td>
<td>25.90</td>
<td>18.70</td>
<td>34.50</td>
</tr>
<tr>
<td>Melanin (%)</td>
<td>0.1% Kakadu Cream</td>
<td>4.40</td>
<td>2.50</td>
<td>4.30</td>
<td>0.80</td>
<td>9.10</td>
</tr>
</tbody>
</table>

Comparative analysis was performed using a parametric test because the data were normally distributed (p>0.05). Data were analyzed using an independent t-test. The results of the comparative analysis are presented in table 2.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Mean±SD</th>
<th></th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosinase (%)</td>
<td>Placebo Cream 0.1%</td>
<td>36.19±4.60</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1% Kakadu Cream</td>
<td>14.99±2.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melanin (%)</td>
<td>Placebo Cream</td>
<td>25.93±4.52</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1% Kakadu Cream</td>
<td>4.40±2.50</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The comparative analysis results showed that the mean tyrosinase expression in the control group was 36.19±4.60%, while in the treatment group, it was 14.99±2.30% (p<0.001). This indicated that the tyrosinase expression between the control and treatment groups was significantly different after two weeks of treatment (p<0.05).

![Image](image2.png)

**Figure 4:** Comparison of tyrosinase expression between groups

![Image](image3.png)

**Figure 5:** Comparison of the amount of melanin between groups

The comparative analysis results showed that the mean amount of melanin in the control group was 25.93±4.52%, while in the treatment group, it was 4.40±2.50%. Comparative analysis using the independent t-test showed that the p<0.001. This indicates that the amount of melanin between the control and treatment groups, after two weeks of treatment is significantly different (p<0.05).
4. Discussion

This study showed a significant difference in tyrosinase expression between the control group and the treatment group. The group was given 0.1% Kakadu cream had a lower mean tyrosinase expression than the control group. Kakadu plum (Terminalia ferdinandiana), which contains ascorbic acid, which is very high, even 500 times higher than blueberries, has a considerable effect on tyrosinase activity. The results of this study are in line with the study by Choi et al. (2009). They compared the inhibitory effect of tyrosinase between the control and treatment groups given ascorbic acid in vitro. The group was given 500 µM, and 1000 µM ascorbic acid had a higher inhibitory effect than the control group.7

Research by Panich et al. (2011) compared the inhibitory effect of ascorbic acid and kojic acid on tyrosinase activity, and it was found that kojic acid with a concentration of 240 µM had an inhibitory effect of 30% while ascorbic acid at the same concentration had an inhibitory effect of 70%.8 Ascorbic acid can stimulate tyrosinase activity, tyrosinase expression and increase the expression of melanogenic regulatory factors, such as tyrosinase-related protein-1 (TRP-1), microphthalmia-associated transcription factor (MITF), and dihydroxy-phenylalanine-chrome-tautomerase (TRP-2). Ascorbic acid also induces mitogen-activated protein kinase (MAPK) phosphorylation. The inhibition of the p38 MAPK pathway by the p38 MAPK inhibitor (10 µM SB203580) will cause suppression of the expression of tyrosinase, TRP-1, and TRP-2 in cells given ascorbic acid.9

This study also found a significant difference in the amount of melanin between the control and treatment groups after two treatment weeks. The group was given 0.1% Kakadu cream had a lower amount of melanin than the control group. This is in line with the study by Choi et al. (2009), which compared the amount of melanin in the control group and the treatment group given ascorbic acid to B16 melanoma cells.7 It was found that the control group had melanin expression of nearly 100%. In contrast, the group was given ascorbic acid of 250 µM, 500 µM, and 1000 µM had lower amounts of melanin, namely 90%, 60%, and 40%, respectively. The results of a study by Panich et al. (2011) also showed that ascorbic acid with a concentration of 120 µM was able to significantly reduce melanin production in melanoma G361 cells exposed to UVA 16 J/cm².8

The anti-melanogenic effect of ascorbic acid is by suppressing reactive quinones produced by the interaction of tyrosinase and L-DOPA, and by suppressing UV light-mediated oxidant formation so that melanin cannot be formed by tyrosinase action until L-ascorbic acid is oxidized.10

UV rays can directly stimulate melanogenesis by suppressing lipids in the plasma membrane of melanocytes so that diacylglycerol is released into the cytoplasm and activating the tyrosinase. UV rays have an indirect effect on melanocytes by inducing keratinocytes to synthesize several paracrine melanocyte factors, for example, basic fibroblast growth factor, endothelin-1, a-melanocyte-stimulating hormone (MSH), and prostaglandin E2 (PGE2), which will stimulate melanocyte proliferation. UV rays can affect the skin by inducing the formation of reactive oxygen species (ROS), which play a role in initiating oxidation reactions during melanogenesis, assisting melanin biosynthesis, and inducing melanocyte proliferation.10

Pigmentation stimulation by UVB radiation was found to be greater than UVA after exposure to tyrosine. In vivo studies showed that UVB radiation has an effect of three to four times greater per unit physical dose (J/cm²) when compared to UVA in inducing erythema, DNA damage, tanning, and skin cancer.11 UVB radiation is also associated with risk factors for nonmelanoma skin cancer, especially squamous cell carcinoma, because it can cause direct damage to DNA. Even though it does not damage the plasma membrane's integrity, low-grade UVB and UVA radiation will still reduce the proliferation of melanocyte cells.11

This study shows the effectiveness of Kakadu cream in overcoming hyperpigmentation after UVB exposure. In the treatment group that received 0.1% Kakadu cream, there were fewer brown hyperpigmented lesions. In contrast, there were more hyperpigmented lesions after UVB exposure in the control group applied with a placebo cream. The results of this study are in line with the research of Hwang et al. (2009). That study showed ascorbic acid could have a depigmentation effect by inhibiting the melanogenic peroxidase-catalyzed reaction in melanocytes. Ascorbic acid can change pigmentation from black to brown and indicate significant changes in the skin with melasma.10

Kakadu cream also contains ellagic acid, which reduces the expression of IL-1β, IL-6, IL-8, monocyte chemotactrant protein-1 (MCP-1), and UVB-induced TNF-α in vitro. Ellagic acid also helps fibroblasts exposed to UVB to block the secretion of metalloproteinases (MMPs) and cause collagen degradation so that wrinkles on the skin become less.12 The study by Shimogaki et al. (2000) showed that with a low concentration of 4 µM, ellagic acid could suppress tyrosinase activity by 38.3% and reduce the increase in the amount of melanin by 54.4%.13 Oral ellagic acid supplementation at a dose of 100-200 mg/day for four weeks can inhibit tyrosinase activity, which is also useful as an anti-melasma.14

Several studies have shown the potential of ellagic acid as an antioxidant and anti-inflammatory such as the ability to protect human keratinocyte cells against oxidative stress and UV-induced apoptosis and inhibit the tautomerase activity of Migration Inhibitory Factor (MIF), which is mediated by a pro-inflammatory response.15,16

Volume 9 Issue 11, November 2020
www.ijsr.net
Licensed Under Creative Commons Attribution CC BY

Paper ID: SR201116161123 DOI: 10.21275/SR201116161123 1048
Besides containing ascorbic acid and ellagic acid, Kakadu plum (Terminalia ferdinandiana) also contains polyphenols and tannins that can suppress tyrosinase expression. According to one study, polyphenols effectively absorb UVB rays in the 290-315 nm spectrum and UVA rays in the 315-400nm spectrum. The inhibitory effect of polyphenols against tyrosinase being the most effective at concentrations of 600 µg/ml.17 Meanwhile, 200 µg/mL tannins reduced intracellular tyrosinase activity and the amount of melanin in melanoma B16 cells, namely 40.3±1.5% and 45.2±1.3%.18

5. Conclusion

Based on this study's results, it can be concluded that Kakadu plum (Terminalia ferdinandiana) cream 0.1% prevented the tyrosinase expression and the amount of melanin in the ultraviolet B- exposed male guinea pig (Cavia porcellus) skin.

6. Suggestion

Further research needs to be done to compare the effectiveness of 0.1% Kakadu cream with the gold standard hyperpigmentation therapy, such as hydroquinone. Long-term research is needed to determine the side effects of using Kakadu cream.

References


Volume 9 Issue 11, November 2020

www.ijsr.net
Licensed Under Creative Commons Attribution CC BY

Paper ID: SR201116161123 DOI: 10.21275/SR201116161123 1049