I. INTRODUCTION

Aging is a natural process that continues to occur with age. Aging is caused by many factors, such as extrinsic and intrinsic factors. The skin is the outermost part of the body so it is prone to external exposure which causes skin aging. Hyperpigmentation is one of the symptoms of skin aging that people often complain about. One of the biggest causes is UV rays exposure [1]. UV rays consist of UVA, UVB, and UVC. UV A light cause an intermediate pigmented darkening which has the
effect of increasing the distribution of melanin that already exists. While UV B exposure cause delayed pigmented
darkening that lead to increase in melanin production, an
increase in the quantity of melanocytes, an increase in the
tyrosinase enzyme and the spread of melanin [2]. UV rays
play a major role in causing skin aging which is characterized
by hyperpigmentation, wrinkles, keratosis, and decreased
skin elasticity. Chronic UV exposure can also cause skin
damage to DNA mutations that lead to skin cancer [3], [4].

The current gold standard for hyperpigmentation therapy is
2-4% hydroquinone. The way this compound works is by
inhibiting the tyrosinase enzyme, causing melanocyte
damage and also accelerating melanosome degradation.
However, long-term use causes side effects such as irritation,
rebound phenomenon, and even ochronosis [5]. Therefore, it
is necessary to find natural ingredients with minimal side
effects as hyperpigmentation therapy.

Black soybean is a potential anti-pigmentation ingredient
because it contains a total of 930.8 mg of phenol, 9277.99 mg
of flavonoids, and 1045.99 mg of tannin. The content of
antioxidants such as flavonoids and total phenol in black
soybeans is higher than yellow soybeans [6]. Phenols,
flavonoids and tannins are antioxidant compounds that
function to neutralize free radicals thereby inhibiting the
initiation of the melanogenesis process. In addition,
flavonoids in black soybeans, namely isoflavones (genistein)
also work to inhibit the tyrosinase enzyme so that it inhibits
the formation of melanin [7]. Black soybeans have been very
popular for their use as medicine. Among Indonesian people,
it is usually fermented into sweet soy sauce.

Previous research has shown that 30% black soybean
extract was able to inhibit the increase of the tyrosinase
enzyme expression and the amount of melanin in the skin of
guinea pigs exposed to UVB light when compared to the base
cream [8]. Another study by [9] examined the extract of black
soybean sprouts which showed that there was an inhibition of
the formation of the tyrosinase enzyme, thereby reducing the
number of skin melanin cell production. The purpose of this
study was to compare the effectiveness of 30% black soybean
extract with the gold standard for pigmentation therapy,
namely 4% HQ cream in preventing the increase in tyrosinase
enzyme expression and the amount of melanin in guinea pig
skin exposed to UV light.

II. METHOD

This study used a post-test only control group design
method with a sample of 30 guinea pigs. The samples were
divided into 3 groups of 10 guinea pigs each. Group P1 was
given basic cream, group P2 was given 4% HQ cream and
group P3 was given 30% black soybean ethanol extract
cream. Each group was applied cream 20 minutes before
exposure and 4 hours after exposure. UVB exposure using
Philips PL-S 9W/01/2P brand. UVB rays is given 3 times a
week, Monday, Wednesday and Friday for 65 seconds with a
distance of 15cm so that the total dose of UVB radiation is
390mJ/cm². On days without exposure, the cream is still
applied 2 times a day. This treatment was carried out for 2
weeks. After that, the guinea pigs were left for 48 hours to
avoid the effects of acute exposure, then were euthanized and
then the skin tissue was taken to make histopathological
preparations. The research was conducted at the Integrated
Biomedical Laboratory Unit, Faculty of Medicine, Udayana
University for 8 months from August 2011-March 2022.

A. Black Soybean Extraction

The extraction technique uses the maceration method,
because it can avoid damage to thermolabile compounds.10
One kilogram of selected black soybeans then washed and
blended. Then 96% ethanol was added in a 1:1 ratio (1000 g
black soybeans: 1000 g ethanol 96%) then macerated in a
dark bottle that was tightly closed for 24 hours at 4 °C. Then
the solution was filtered using Whatman paper no. 1 and the
filtrate was evaporated for 20 minutes. A thick extract was
obtained which was the result of evaporation and then
weighed, labeled and stored in a dark bottle at 4 °C.

B. Black Soybean Ethanol Extract Cream 30%

The basic ingredient formulation of the cream is 305 3%
sepiol as an emulsifier mixed into water for 5 minutes, then
add 2% lanol, 2% dimethicon and 0.5% phenoxyethanol,
continue mixing until the ingredients are in creamy state. To
get a 30% concentration of black soybean extract cream
required 30 grams of extract in a total mixture of 100 grams
of cream.

C. Examination of Tyrosinase Enzyme Expression by
Immunohistochemical Staining

Assessment of tyrosinase enzyme expression was carried out
by recording the preparation using the Optilab Viewer
which was connected to a CX40 microscope that produce
JPEG images, then manual calculations were performed
using Image Raster software. The expression of the tyrosinase
enzyme was shown by melanocyte cells in the epidermis
with brown cytoplasm. The expression of the tyrosinase
enzyme was measured by counting melanocyte cells in the epidermis
that expressed the tyrosinase enzyme divided by the total
edipidermal cells multiplied by 100% [11].

D. Examination of the Amount of Melanin with Mason-
Fontana

The preparations were obtained then photographed with an
Optilab Pro camera and an Olympus CX40 microscope using
400 times magnification. Each preparation was photographed
three times in the left, middle, and right sides. Then the photos
are saved into a JPEG file format. The calculation of the
amount of melanin was carried out using a digital rapid
analysis method. This procedure uses Adobe Photoshop CS3
software version 9.0. In the Adobe Photoshop CS3 program,
melanin appears black using the magic wand function. Then
choose a pixel other than black by using the inverse function,
then use the delete function to delete it so that the remaining
image is only black pixels. The amount of melanin was
analyzed as the percentage of the pixel area of the melanin
that is black compared to the pixel area of the entire tissue
[12].

E. Data Analysis

After the data being collected, it is processed with SPSS
17.0 for windows. Numerical data consists of three unpaired
groups. The normality test of the data used the Shapiro-Wilk
test, while the homogeneity test used Levene's test. The mean
difference between the three groups was carried out by
statistical tests using the one way Anova test, then to determine the magnitude of the differences in each group, the Least Significant Difference-test (LSD) was performed.

III. RESULTS

From the macroscopic picture of the three groups, the pigmentation was not significantly different between the 30% black soybean extract cream group (P3) and the 4% HQ cream group (P2) while the base cream group (P1) showed the most pigmentation among the three groups. This is also reflected in the microscopic image, where the tyrosinase enzyme expression and the amount of melanin in the 30% black soybean extract group (P3) did not differ significantly from the 4% HQ group (P2), while the highest of tyrosinase enzyme expression and the amount of melanin was in the base cream group (P3).

![Fig. 1. Macroscopic picture of guinea pig skin before and after treatment. There was no significant difference in pigmentation between the 30% black soybean extract cream group (P3B) and the 4% HQ cream group (P2B) while the base cream group (P1B) showed the most pigmentation among the three groups.](image1)

Descriptive analysis showed the average expression of the tyrosinase enzyme and the amount of melanin in the base cream group (P1) (25.78±5.392% and 12.080±3.150), the HQ group 4% (P2) (9.690±2.176% and 2.18±1.117), and the 30% black soybean extract cream group (P3) (12.880±3.327% and 3.16±1.486). Normality test with Shapiro-wilk test obtained P> 0.05 which indicates that the data is normally distributed. While the data homogeneity test using Levene's test was obtained p= 0.05 which indicates homogeneous data.

The significance analysis using one way Anova on tyrosine enzyme expression and the amount of melanin was obtained (F= 48,501, p= 0.000) and (F=59,567, p=0.000). This indicated that the three groups had a very significant difference in the mean expression of the tyrosinase enzyme and the mean amount of melanin (p<0.001).

Furthermore, to find out the differences between groups, the Least Significance Difference (LSD) test was carried out. The result was that there was a significant difference between the base cream group (P1) and the other groups (p<0.01), but there was no significant difference between the 4% hydroquinone group (P2) and the 30% black soybean extract cream group (P3) (p >0.05).

![Fig. 2. Expression of guinea pig skin tyrosinase enzyme in the three groups. In the cross section of epidermal skin tissue, the expression of the tyrosinase enzyme in epidermal melanocyte cells is visible from the brown melanocyt e cell cytoplasm (arrow). The expression of the tyrosinase enzyme in the 30% black soybean extract group (P3) was not significantly different from the 4% hydroquinone group (P2) while in the base cream group (P1) the expression of the tyrosinase enzyme was the highest among the three groups.](image2)

![Fig. 3. The amount of guinea pig skin melanin in the three groups. In the cross section of the epidermal skin above, the melanin pigment is visible as seen from the black pixels in the Masson-Fontana staining. Melanin pigment in the 30% black soybean extract group (P3) was not significantly different from the 4% hydroquinone group (P2) while the base cream group (P1) showed the highest amount of melanin among the three groups.](image3)
and the amount of melanin did not significantly differ. In the analysis, it can be seen that the macroscopic images, microscopic images, and statistical analysis showed that the acute effects of UVB rays exposure were avoided. From the results, guinea pigs were allowed to stand for 48 hours after 2 weeks of exposure.

### Table I: Descriptive Analysis Result of Tyrosinase Enzyme Expression and the Amount of Melanin

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>n</th>
<th>Mean±SD</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosinase enzyme expression</td>
<td>Base cream (P1)</td>
<td>10</td>
<td>25.7±8.5392</td>
<td>24.2</td>
<td>18.3</td>
<td>35.1</td>
</tr>
<tr>
<td></td>
<td>4% HQ (P2)</td>
<td>10</td>
<td>9.690±2.176</td>
<td>9.7</td>
<td>6.4</td>
<td>13.4</td>
</tr>
<tr>
<td></td>
<td>30% Black soybean extract cream (P3)</td>
<td>10</td>
<td>12.880±3.327</td>
<td>12.650</td>
<td>7.5</td>
<td>17.7</td>
</tr>
<tr>
<td>The amount of melanin</td>
<td>Base cream (P1)</td>
<td>10</td>
<td>12.080±3.150</td>
<td>12.7</td>
<td>6.7</td>
<td>16.5</td>
</tr>
<tr>
<td></td>
<td>4% HQ (P2)</td>
<td>10</td>
<td>2.18±1.117</td>
<td>2.05</td>
<td>0.5</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>30% Black soybean extract cream (P3)</td>
<td>10</td>
<td>3.16±1.486</td>
<td>3.25</td>
<td>0.9</td>
<td>5.7</td>
</tr>
</tbody>
</table>

### Table II: Comparative Analysis of Tyrosinase Enzyme Expression and the Amount of Melanin Between Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Mean Difference</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosinase enzyme expression</td>
<td>Base cream (P1)</td>
<td>16.0900</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>4% HQ (P2)</td>
<td>12.9000</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>30% Black soybean extract cream (P3)</td>
<td>-3.1900</td>
<td>0.076</td>
</tr>
<tr>
<td></td>
<td>Base cream (P1)</td>
<td>-12.9000</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>4% HQ (P2)</td>
<td>3.1900</td>
<td>0.076</td>
</tr>
<tr>
<td></td>
<td>30% Black soybean extract cream (P3)</td>
<td>2.02704</td>
<td>0.000</td>
</tr>
<tr>
<td>The amount of melanin</td>
<td>Base cream (P1)</td>
<td>1.71889</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>30% Black soybean extract cream (P3)</td>
<td>1.71889</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Base cream (P1)</td>
<td>-2.02704</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>4% HQ (P2)</td>
<td>0.30816</td>
<td>0.135</td>
</tr>
<tr>
<td></td>
<td>30% Black soybean extract cream (P3)</td>
<td>-0.30816</td>
<td>0.135</td>
</tr>
</tbody>
</table>

This can be happening because black soybeans contain total phenols, flavonoids, and tannins which have functions as antioxidants. Antioxidant work to neutralize free radicals thereby inhibiting the initiation of the melanogenesis process [13]. Flavonoids work directly to scavenge reactive oxygen species and chelate free radicals immediately by donating their hydrogen atoms or by transferring single electrons [14]. It is also known that black soybeans contain a specific isoflavone type flavonoid, namely genistein, which functions to inhibit the tyrosinase enzyme so that it inhibits the hydroxylation of tyrosine to DOPA. Black soybeans also contain a protease inhibitor called the Bowman-Birk protease inhibitor and soybean trypsin. This protease inhibitor works to prevent the transfer of melanosomes to keratinocytes [15], [16]. These things are the advantages of black soybean in its effect on preventing the increase in the amount of melanin.

By inhibiting the formation of melanin, indirectly inhibits the formation of melanin so that it affects in the amount of melanin. The mechanism of action of the active substances in black soybeans are same as hydroquinone. Hydroquinone works by inhibiting tyrosinase thereby inhibiting the increase in the amount of melanin [17].

The results of this study were similar to the results of previous studies on black soybean sprout extract which showed that extract was inhibited the formation of the tyrosinase enzyme, thereby reduced the number of skin melanin cell production [9]. Research by [8] also proved that the administration of 30% black soybean extract succeeded in inhibiting the tyrosinase enzyme expression and the amount of melanin when compared to the control group. Previously another study was conducted by [18], researched the compound genistein showed that genistein was successful in inhibiting pigmentation on the skin of guinea pigs so that it could be used as a therapy for hyperpigmentation induced by...
UVB rays. In addition, research by [19] using gemitir flower extract containing flavonoid active substances managed to prevent an increase in the amount of melanin. This flavonoid active substance is also found in black soybeans.

This study has proven that administration of 30% black soybean ethanol extract cream was as effective as 4% hydroquinone cream in inhibited the increased in tyrosinase enzyme expression and the amount of melanin in guinea pig skin exposed to UVB rays. We hoped that someday in the future black soybean extract can be used as an alternative material for hyperpigmentation therapy, which has been played by hydroquinone as the gold standard.

V. CONCLUSION

The conclusion of this study was that administration of 30% black soybean ethanol extract cream inhibited the tyrosinase enzyme expression and the amount of melanin as effective as 4% hydroquinone on guinea pig skin exposed to UVB light. In the end, we hoped that this research can also be applied to humans, but further research is needed, including the safety index and clinical trials in humans so that the results of this study are really useful.

REFERENCES


