Administration Of 30% Robusta Coffee (*Coffea canephora*) Silverskin Ethyl Acetate Fraction Cream Inhibited The Increase Of MITF Expression And Melanin Amount In The Skin Of Male Adult Guinea Pig (*Cavia porcellus*) Exposed To Ultraviolet B Ray

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Abstract

Background: Melanin is produced through melanogenesis which involves several molecular processes with microphthalmia-associated transcription factor (MITF) as the main regulator of melanin synthesis. Hyperpigmentation is one of aging sign that happens due to melanin buildup in the skin. Hyperpigmentation can be caused by many factors, and one of the most frequent cause is ultraviolet (UV) ray exposure. Robusta coffee silverskin is a thin layer on a green coffee bean that is detached from the bean through roasting process, and is a waste product of coffee production. However, coffee silverskin contains high amount of phenolic and flavonoid compounds, and is categorized as strong antioxidant. The purpose of this study was to prove that administration of 30% Robusta coffee silverskin ethyl acetate fraction cream inhibited the increase of MITF expression and melanin amount in the skin of male adult guinea pig exposed to UVB ray.
Methods: This study used randomized post-test only control group design. Subjects used in this study were healthy male guinea pigs with age ranging from 3-4 months and average weight 300-350g. Total samples used in this study were 36 guinea pigs divided evenly into two groups, Group K that were only exposed to UVB ray, and Group P that were exposed to UVB ray and 30% Robusta coffee silverskin ethyl acetate fraction cream. Total UV ray used during two weeks were 390mJ/cm^2. 30% Robusta coffee silverskin ethyl acetate fraction cream was applied twice a day on guinea pigs at the days they were not exposed to UVB ray, and at the days they were exposed to UVB ray, the cream was applied 20 minutes before and 4 hours after the exposure. MITF expression was examined under the microscope with MITF antibody kit, and melanin amount was examined with Masson-Fontana staining.
Results: Result of the experiment showed higher MITF expression and melanin amount in the Group K.

Comparison analysis with 2 independent samples t-test shows average MITF expression in the Group K and Group P 27.833 vs 15.422 % MITF, and 16.828 vs 7.822 % pixel melanin for the melanin amount. The differences between two variables were meaningful as the *p* value <0.001.
Conclusions: Conclusion for this experiment is that 30% Robusta coffee silverskin ethyl acetate fraction cream inhibited the increase of MITF expression and melanin amount in adult male guinea pigs exposed to UVB.

Keywords: Robusta coffee silverskin ethyl acetate fraction; MITF; Melanin; Ultraviolet B Ray

1. Introduction

The skin is the biggest and the most visible organ of the human body. Similar to other body parts, the skin is also affected structurally and physiologically by the aging process. Aging of the skin manifests through several signs, such as the decrease of skin's elasticity and moisture, emergence of wrinkles, and hyperpigmentation. Hyperpigmentation is a condition where there is a buildup of melanin on the skin, either due to increased production or decreased melanin degradation. Exposure to ultraviolet ray is known to be one of the leading causes of skin damage that accelerates aging process, known as photoaging. Among the types of ultraviolet rays, ultraviolet B penetrates the skin up till the base layer of epidermis where melanin synthesis occurs (Laberge et al., 2020)

Process of melanin synthesis called melanogenesis, involves series of molecular events with *Microphthalmia-associated Transcription Factor* (MITF) as the main regulator of the process (Idana et al., 2022). Skin exposure to ultraviolet B produces free radicals such as Reactive Oxygen Species (ROS) which damages DNA of the skin. This damage activates MITF in the melanocyte, and with the increase of MITF expression, more melanin is produced and deposited to the keratinocyte of epidermis, resulting in hyperpigmentation. Even though this process is protective in nature, uneven distribution of melanin in the skin can cause esthetic issues which may impact the patient's quality of life. (Hida et al., 2020; Kremslehner et al., 2020).

Robusta coffee (*Coffea canephora*) silverskin is a thin layer on a coffee bean that is detached from the bean during the roasting process. While coffee silverskin is considered as waste product of coffee production, it contains antioxidant properties such as phenolic compounds and flavonoids. Phenolic compounds such as chlorogenic acid, coumarate acid, and ferulic acid is present in coffee silverskin, and is known to have anti-melanogenic effects by degrading MITF, reducing the activity of cAMP/CREB signaling pathway in melanogenesis, and inducing melanocyte death. Another antioxidant present in coffee silverskin is flavonoid, which works by stabilizing reactive oxygen compounds to produce less reactive radicals (Xuan et al., 2019; Iriondo-DeHond et al., 2019).

2. Methods:

A randomized post-test-only control group design was conducted among 36 male Guinea pigs for 21 consecutive days at the Laboratory Animal Unit, Department of Pharmacology, Faculty of Medicine, Udayana University. The Guinea pigs were divided into two groups, namely: control group (K) that is only exposed to UVB, and treatment group (P) that is exposed to both UVB and 30% Robusta coffee silverskin ethyl acetate fraction cream.

All guinea pigs were adapted for 7 days before any treatment was started. Both groups are exposed to 65 mJ/cm^2 UVB every Monday, Wednesday, and Friday for two weeks, with total UVB energy 390 mJ/cm^2. UVB is exposed for 65 seconds during each session. Guinea pigs in the treatment group (P) were applied with 30% Robusta coffee silverskin ethyl acetate fraction cream 20 minutes before any exposure to UVB, 4 hours after exposure to UVB, and twice a day during days without UVB exposure. Throughout the study period, all guinea pigs were given equally adequate amount of food and water. Rest period for 2 days were given to the guinea pigs after the last day of UVB exposure to prevent any bias caused by acute effects of UVB exposure. Skin biopsies with thickness of approximately 5mm and surface area of 1x1cm^2 were made, then sent to the laboratory for histological examination.

MITF expression was examined through immunohistochemistry method using anti-MITF primary antibody kit. The examination was done at the Department of Histology, Faculty of Medicine, Udayana University. Melanin amount was examined and calculated through immunohistochemistry method using Masson-Fontana staining at the Department of Histology, Faculty of Medicine, Udayana University.

Acquired data were analyzed statistically using two independent samples t-test for MITF expression and melanin amount, with SPSS version 25.0 program. Results for mean, standard deviation, and significance test were shown. p-value of less than 0,05 was obtained, thus the result for MITF expression and melanin amount is statistically significant.

3. Results

The macroscopic and histological examination of MITF expression and melanin amount from each group of Guinea pigs were shown below. The results of MITF expression showed higher mean value in group K (27.833±6.31) than group P (15.422±8.26). Similarly, higher mean value for melanin amount was observed in group K (16.828±5.44) followed by group P (7.822±6.14). Data of MITF expression and melanin amount from each group were tested for normality and homogeneity through Saphiro-Wilk Test and Levene Test. Both data were normally distributed with $p>0.05$, and both data were homogenous with $p>0.05$. Analysis of the results was done with independent two samples t test which showed significant difference between group K and group P ($p<0.001$).

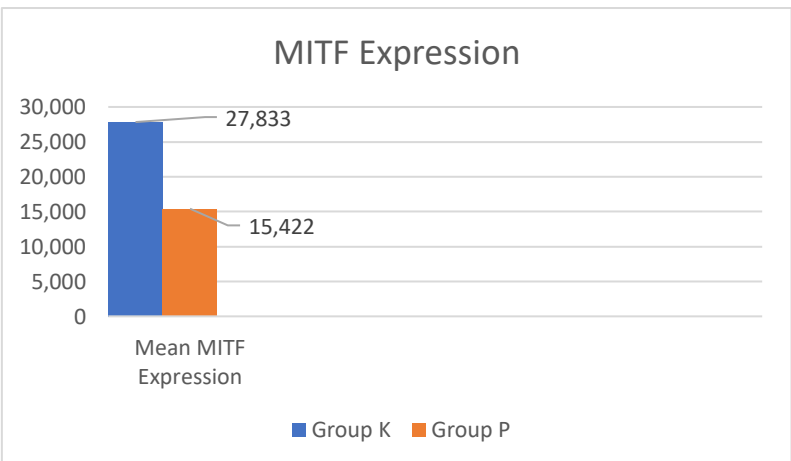


Figure 1. The Mean of MITF expression of Guinea pigs in Group K and Group P

Table 1. The Mean of MITF expression of Guinea pigs in Group K and Group P

Variable	Group	n	Mean	SD	Median	Min	Max
MITF	K	18	27.833	6.31	28.650	17.5	37.5
Expression	P	18	15.422	8.26	15.150	2.2	28.4

Footnotes:
K : Control group (UVB exposure only group)
P : Treatment group (UVB exposure + 30% Robusta coffee silverskin ethyl acetate fraction cream

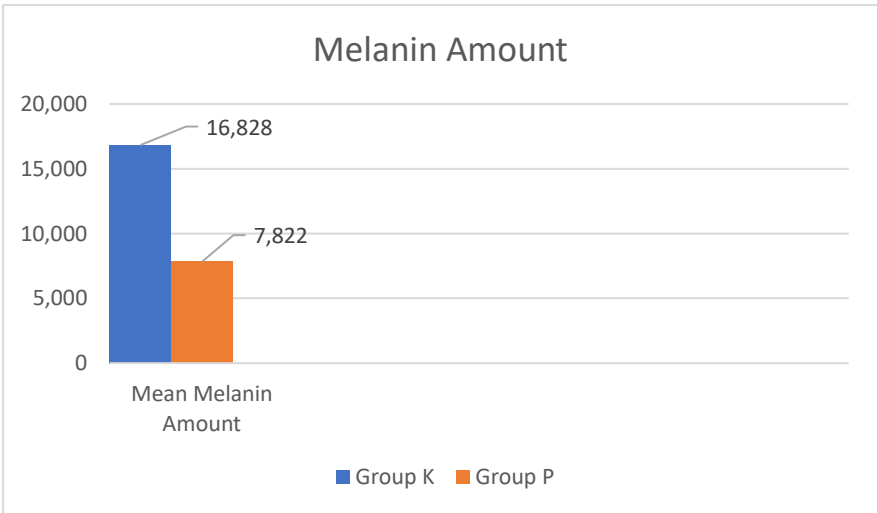


Figure 2. The Mean of Melanin Amount of Guinea pigs in Group K and Group P

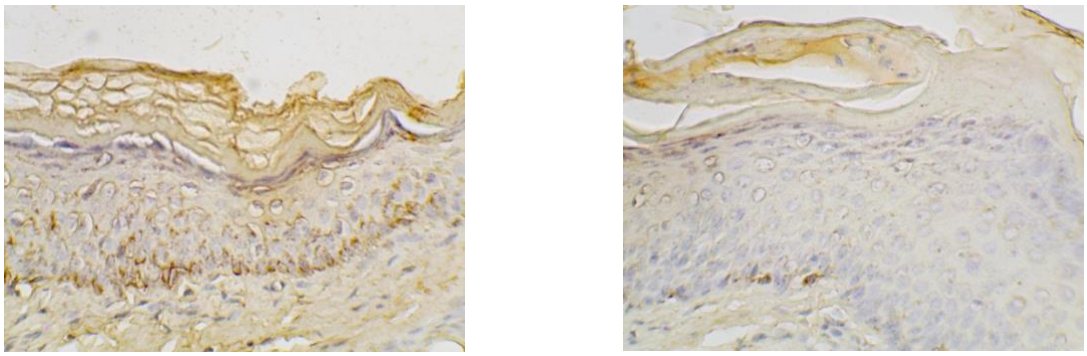
Table 2. The Mean of Melanin Amount of Guinea pigs in Group K and Group P

Variable	Group	n	Mean	SD	Median	Min	Max
Melanin	K	18	16.828	5.44	17.950	8.3	25.1
Amount	P	18	7.822	6.14	6.0	0.9	19.7

Footnotes:
K : Control group (UVB exposure only group)
P : Treatment group (UVB exposure + 30% Robusta coffee silverskin ethyl acetate fraction cream

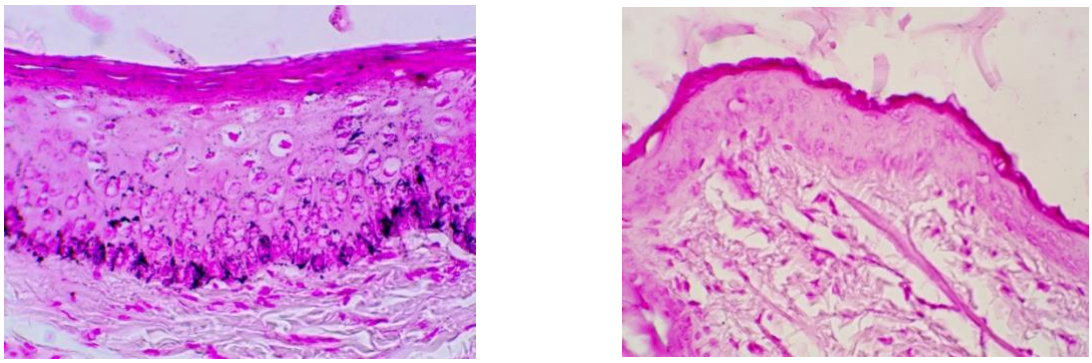
The results were similar for MITF expression and melanin amount variables where the mean value of group K (MITF expression 27.833±6.31; Melanin amount 16.828±5.44) was higher than group P (MITF expression 15.422±8.26**;** Melanin amount 7.822±6.14). Data from the two variables were tested for normality and homogeneity with Saphiro-Wilk Test and Levene's Test. The results were analyzed using independent two samples t test which showed significant difference between group K and group P ($p<0.001$).

Histological examination of MITF expression and melanin amount was shown in Figure 3 and Figure 4 below. MITF expression was assessed with immunohistochemistry method using primary anti-MITF antibody kit, and melanin amount was assessed with immunohistochemistry method using Masson-Fontana staining. Macroscopically, the skin of guinea pigs from group K displayed wider and darker area of hyperpigmentation compared to the guinea pigs from group P, shown in figure 5.



(a) (b)

Figure 3. The microscopic histology preparate of MITF expression (dark brown spots) in the skin of Guinea pig, using primary anti-MITF antibody kit. (Light microscope, 10x 40 magnification). Group (a) K = UVB exposure; (b) P = UVB exposure and 30% Robusta coffee silverskin ethyl acetate fraction cream 30%



(a) (b)

Figure 4. The microscopic histology preparate of melanin amount (black spots) in the skin of Guinea pig, using Masson-Fontana staining. (Light microscope, 10x 40 magnification). Group (a) K = UVB exposure; (b) P = UVB exposure and 30% Robusta coffee silverskin ethyl acetate fraction cream 30%

(a) (b)

Figure 5. The macroscopic appearance of the skin of Guinea pig from group K displayed darker and wider area of hyperpigmentation. Group (a) K = UVB exposure; (b) P = UVB exposure and 30% Robusta coffee silverskin ethyl acetate fraction cream 30%.

4. Discussion

This study proved that 30% Robusta coffee (*Coffea* canephora) silverskin ethyl acetate fraction cream inhibited the increase of MITF expression and melanin amount in the skin of male adult Guinea pig exposed to ultraviolet B ray.

The result of MITF expression showed higher mean value in group K (27.833±6.31) compared to group P (15.422±8.26). Similarly, the melanin amount was higher in group K (16.828±5.44) than in group P (7.822±6.14). The data acquired for each variables were analyzed using independent two samples t test, and showed significant difference between group K and P ($p<0.001$).

The histological analysis for MITF expression and melanin amount was shown in Figure 3 and Figure 4. MITF expression was examined with primary anti-MITF antibody kit, and melanin amount with Masson-Fontana staining. Both variables were examined with immunohistochemistry, using a light microscope with 10x 40 magnification. Macroscopically, the skin of Guinea pigs from group K showed darker and wider area of hyperpigmentation.

Chronic exposure of UVB ray causes DNA damage in the skin. Melanin synthesis is triggered to counteract this damage, since melanin has photoprotective attribute for the skin. This process involves a series of molecular events, with MITF as the main regulator of melanin synthesis. Increase in MITF expression stimulates the melanosomes to produce and distribute more melanin into keratinocyte, thus increasing the melanin amount also (Rachmin et al., 2020; Lan et al., 2021). This buildup of melanin in the skin causes hyperpigmentation, and is one of photoaging signs due to UV exposure.

Phytochemical examination of Robusta coffee (*Coffea canephora*) silverskin ethyl acetate fraction shows IC50 result of 93.8mg/L, therefore categorized as powerful antioxidant, with Flavonoids 749.582mg QE/100mL, and Phenols 2688.68mg GAE/100mL. Antioxidant mechanism of Flavonoid is by binding directly to any free radicals to create a more stable and less reactive radicals. Phenol has anti-melanogenic traits by mediating MITF degradation, decreasing cAMP/CREB signaling pathway activity, or incites melanocytes apoptosis (Iriondo-deHond et al., 2021).

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This paper presents Administration Of 30% Robusta Coffee (*Coffea canephora*) Silverskin Ethyl Acetate Fraction Cream Inhibited The Increase Of MITF Expression And Melanin Amount In The Skin Of Male Adult Guinea Pig (*Cavia porcellus*) Exposed To Ultraviolet B Ray.

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