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Wamena Arabica Coffee (Coffea Arabica) Bean Extract Cream Inhibits The Increase Of Tyrosynase And Melanin Levels In Guinea Pig (Cavia Porcellus) Exposed To UVB Lights

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ABSTRACT

Introduction: Most of the aging process in the skin occurs due to exposure to sunlight, especially UV-B light, resulting in an increase in the activity of the tyrosinase enzyme and the amount of melanin which will cause hyperpigmentation of the skin. Antioxidants are substances that can provide protection from endogenous and exogenous oxidative stress by free radicals. The purpose of this study was to prove that Wamena roasted Arabica coffee bean extract cream can inhibit the enzymes tyrosinase and melanin in guinea pig exposed to UV-B lights. ***Methods:*** The design of this study was an animal experimental design with a randomized post-test only control group. The subjects of the study were 30 guinea pigs divided into 3 groups: group 1 as control, group 2 with placebo cream and group 3 was with 3% Wamena arabica coffee bean extract cream. The group 2 and 3 were exposed to UV-B light for 2 weeks. The skin of the guinea pig was excised for examination of the levels of the enzymes tyrosinase and melanin. ***Result:*** The average tyrosinase and melanin levels in control group were 19.891% and 4.62%, treatment group 2 was an average of 42.699% and 9.26% and treatment group 3 was 23.886% and 4.87%. ***Conclusion:*** Arabica coffee (Coffea arabica) bean extract cream roasted Wamena 3% inhibits the tyrosinase enzyme levels and inhibits the melanin in guinea pigs (Cavia porcellus) which are exposed to ultraviolet B light.

Keywords: Wamena Arabica coffee; Tyrosynase; Melanin; UVB lights

INTRODUCTION

The aging process of the skin most commonly occurs due to exposure to sunlight, especially UV-B rays which are characterized by increased activity of the tyrosinase enzyme and the amount of melanin which will cause hyperpigmentation of the skin.[1] Darkening of one part of the skin is a physiological body mechanism that functions to prevent and protect against damage to the top layer of the skin, including the layer underneath as a result of exposure to ultraviolet rays. The formation of melanin is considered a mechanism for protecting the skin from ultraviolet rays.[1,2]

Tyrosinase is an enzyme that catalyzes the oxidation of L-tyrosine to 3,4-dihydroxyphenylalanine (L-DOPA) and to DOPAquinone, which important in the creation of melanin. In the process of melanin synthesis, the tyrosinase enzyme is the most important enzyme because this enzyme has a role as a precursor for tyrosine initiation.[3] In addition, exposure to ultraviolet light for a long time will result in excessive production of melanin which is also known as skin hyperpigmentation.[4]

Antioxidants are substances that can provide protection from endogenous and exogenous oxidative stress by free radicals. Phytochemical results of roasted Arabica coffee from Wamena (Coffea arabica) contain a combination of antioxidants, such as: polyphenolic compounds like alkaloids, flavonoids, terpenoids, saponins, and tannins. One of the polyphenol compounds found in coffee in large quantities is chlorogenic acid.

The purpose of this study was to prove that Wamena roasted Arabica coffee bean extract cream can inhibit the tyrosinase enzymes and melanin in guinea pig exposed to UV-B lights.

METHODS

This study was an experimental post-test only control group design using guinea pig (Cavia porcellus), aged 12-16 weeks, with body weight of 300-350 grams. The material used in this study was Wamena arabica coffee bean extract.

The sample was divided into 3 groups: control group, treatment group 1 (P1) which received UV light exposure and placebo cream 2x a day, and treatment group 2 (P2) which received UV light and 3% Wamena arabica coffee bean extract. The treatment group was exposed to UV light three times a week for four weeks. Then the back skin tissue was taken and examined for the amount of melanin using digital analysis and examined for tyrosinase enzyme levels. Data is recorded and analyzed.

RESULTS

A total of 30 subjects were divided into 3 groups (10 subjects each). The first group was the control group without exposure to UV-B lights and to any cream, the second group was the control group (treated by exposure to ultraviolet B light and placebo), the third group was the treatment group (treated by exposure to ultraviolet B light and coffee bean extract cream). arabica roasted wamena 3%).

Figure 1 shows the guinea pig skin as a control that was not exposed. It can be seen that the guinea pig skin does not hyperpigmented. In Figure 2, the group that was given basic cream and exposed to UVB light had more black-brown hyperpigmentation when compared to the treatment group that was exposed to UVB light and applied 3% roasted Arabica coffee bean extract cream Wamena showed fewer brown hyperpigmented lesions.

FIGURE 1: Skin of guinea pigs as a control.

FIGURE 2: Guinea pig skin after treatment.

After two weeks of treatment, a skin tissue biopsy was taken from the back of the guinea pig. This examination was performed with Masson-Fontana stain.

An overview of melanin in guinea pig skin epidermal tissue can be seen in Figure 3.

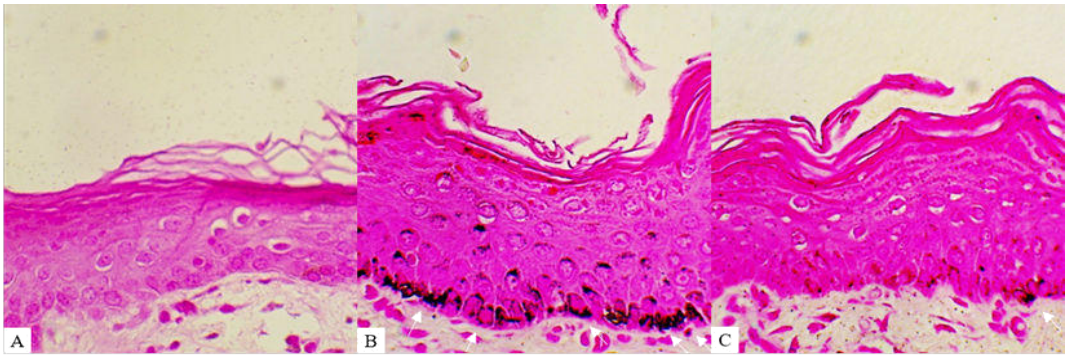

FIGURE 3: The biopsy of the skin Melanin With Masson Fontana Staining.
(A : Control, B: Base Cream, C: Coffee Extract Cream 3%)

The results of the analysis showed that the average Tyrosinase level in the control group was 19.891 nmol/mL (95% CI 19.789 – 19.992), in the group 2 with placebo cream was 42.669 nmol/mL (95% CI 42.18 – 43.217), and in the group 3 with exposure to UVB light and administration of 3% Wamena arabica coffee bean cream obtained an average of 23.886 nmol/mL (95% CI 23.124 – 24.647).

The average amount of melanin in the control group was 4.62 (95% CI 0.994 – 8.246) with a standard deviation of 5.068. Meanwhile, the average amount of melanin in the treatment group with exposure to UVB rays and administration of 3% wamena arabica coffee bean cream was 4.87 (95% CI 1.716 – 8.024) (Table 1).

TABLE 1: Distribution of Tyrosinase Levels and Melanin.

Variables	Group	Mean	SD	Minimal – Maximal	95% CI	p-value
Tirosinase levels (nmol/mL)	Control	19,891	0,141	19,728 – 20,054	19,789 – 19,992	<0,001
	Placebo	42,669	0,725	41,323 - 43,397	42,18 – 43,217	
	Treatment	23,886	1,063	22,527 – 24,81	23,124 – 24,647	
Melanin levels	Control	4,62	5,068	0,8 – 16,3	0,994 – 8,246	0,035
	Placebo	9,26	4,564	1,9 – 17,4	5,995 – 12,525	
	Treatment	4,87	4,408	1,2 – 14,3	1,716 – 8,024	

Statistical test results on Tyrosinase levels obtained a P value of 0.001 meaning that at alpha 5% there was a significant difference in the average Tyrosinase levels in the treatment group. Based on statistical tests on the amount of melanin, a value of p = 0.035 was obtained so that it could be concluded that at alpha 5% there was a significant average difference in the amount of melanin in the three treatment groups. Comparison of the average Tyrosinase levels and the amount of melanin between groups is presented in the graph as follows:

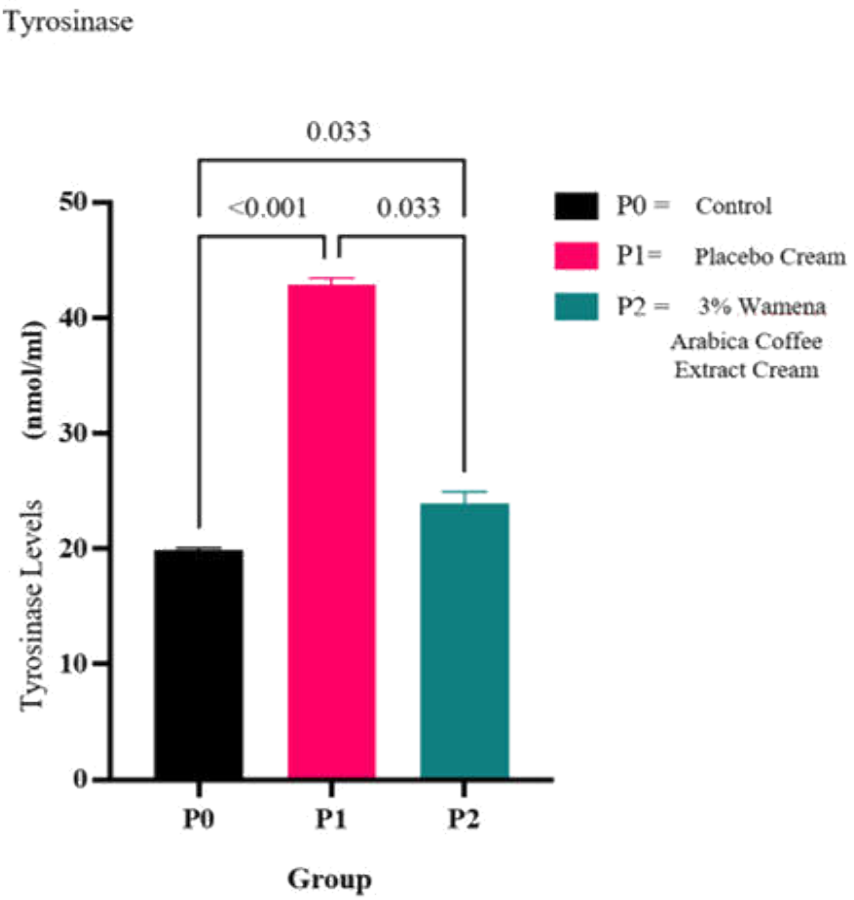

FIGURE 4: Comparison of Average Tyrosinase Levels in Each Group.

LSD post hoc test was carried out to determine differences in tyrosinase levels between groups. It was seen that the placebo cream group had significantly higher tyrosinase levels compared to the placebo group and the treatment group ($p<0.05$).

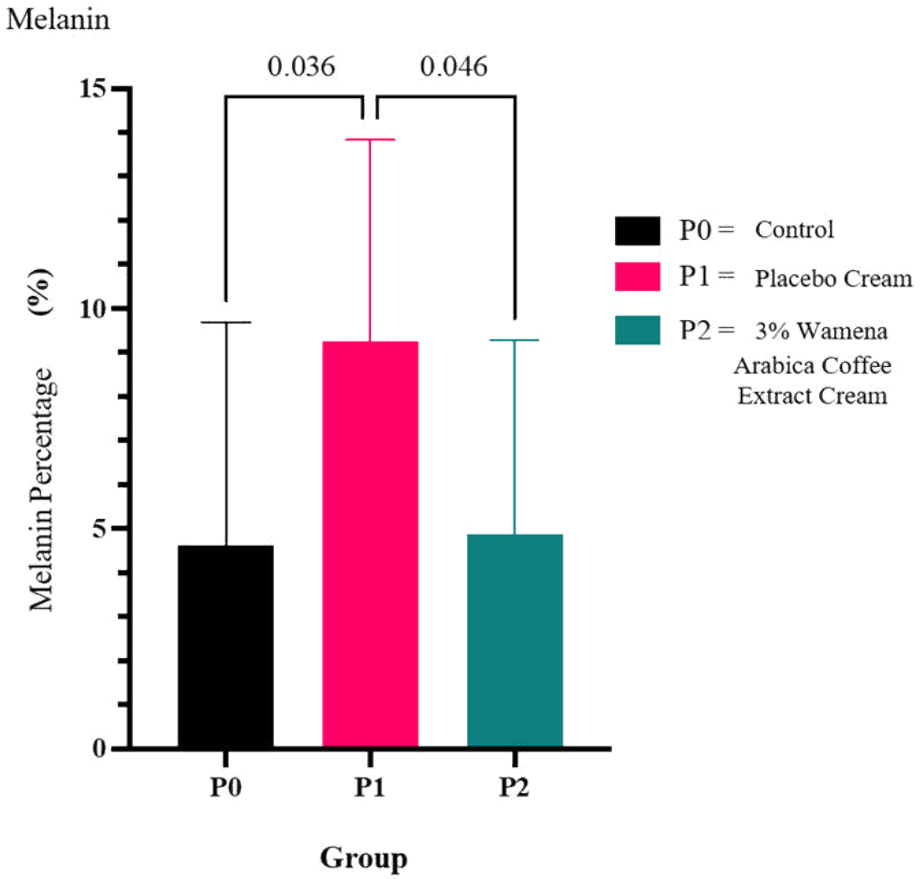

FIGURE 5: Comparison of the Average Amount of Melanin in Each Group.

DISCUSSION

The process of melanogenesis is due to increasing activity of the tyrosinase enzyme which catalyzes the melanogenesis reaction. In addition, the activation of DNA transcription factors and activation of the Mitogen-activated Protein Kinase (MAPK) pathway caused by increased production of Reactive Oxygen Species (ROS) due to exposure to UVB rays are also believed to play a role in increasing melanin production.[6]

Several previous studies stated that Arabica coffee beans *(Coffea arabica)* contain flavonoid compounds, phenol compounds with the main composition chlorgenic acid, and alkaloid compounds such as caffeine.[7] The test results showed that the phytochemical compounds identified in roasted Wamena arabica coffee bean extract had antioxidant activity with an IC50 value of 107.97 ppm.[8] The lower the IC50 value of a tyrosinase inhibitor, the stronger the power of the inhibitor in inhibiting the increase in tyrosinase and melanin enzymes.[9]

Previous research stated that antioxidant agents, especially phenolic compounds contained in Arabica coffee beans have the ability to reduce the synthesis of the tyrosinase enzyme by affecting the expression of the tyrosinase gene. In addition, antioxidant agents can reduce the activity of the tyrosinase enzyme by inhibiting the oxidation of tyrosine to melanin.[10] The higher the dose of coffee bean cream given, the tyrosinase enzyme levels in test animals exposed to UVB light will decrease. The inhibition of tyrosinase enzyme levels is caused by the content of antioxidant agents in Arabica coffee beans. Increasing levels of the tyrosinase enzyme is inhibited by the mechanism of penetration and neutralization of ROS.[11]

Antioxidant compounds such as flavonoids, phenols and alkaloids contained in arabica coffee bean extract can penetrate and neutralize ROS. Antioxidants protect cells from free radical damage by donating a free electron to a free radical or unstable electron to becomes stable and stops the chain reaction and prevents damage to lipids, proteins and DNA .[12]. The mechanism of penetration and neutralization of ROS by antioxidants can also be accomplished by capturing radical compounds such as superoxide (O2-), hydrogen peroxide (H2O2) and hydroxyl radicals (OH). In addition, antioxidants are able to bind metal ions such as iron and copper which play a role in oxidative reactions. By binding to metal ions, the antioxidants stop involving metal ions and prevent the formation of oxygen radicals. In general, natural antioxidants can act as reducing agents, free radical scavengers, metal chelating agents, and absorbers for the formation of oxygen radicals.[13]

Application of Wamena roasted Arabica coffee bean cream showed effectiveness in inhibiting the increase in the amount of melanin in guinea pig skin exposed to UVB rays. The mechanism of melanogenesis is inhibited by antioxidant agents through several mechanisms, namely direct inhibition of the melanogenesis process, inhibition of DNA transcription factors, and inactivation of the mitogen-activated protein kinase (MPAK) pathway.[14]

CONCLUSION

Wamena Arabica coffee bean extract cream (*Coffea arabica*) 3% inhibited the increase in tyrosinase enzyme levels and inhibited the melanin in guinea pigs (*Cavia porcellus*) which were exposed to ultraviolet B light.

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