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Glycine max (L) Merrit Extracts Inhibited the Enhancement of Tyrosinase Expression and the Increase in the Amount of Melanin on Cavia Porcellus Skin that is exposed to Ultraviolet B

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Abstract: ***The process of skin aging due to exposure to ultraviolet B (UVB) light is characterized by an increase in the expression of the enzyme Tyrosinase and the amount of melanin in the skin. Whitening creams used as anti-hyperpigmentation skin containing hydroquinone and mercury cause negative effects on the skin such as irritation, rebound phenomenon, and ochronosis. Black soybean (Glycine max (L) Merrit) is a type of legume that has active ingredients with the potential to inhibit the melanogenesis process by inhibiting the increase in the expression of the enzyme Tyrosinase. The objective of this study is to demonstrate that the application of black soybean extract cream inhibited the increase in Tyrosinase enzyme expression and the amount of melanin in guinea pigs exposed to UVB. This study used a randomized posttest only control group design. Subjects were guinea pigs (Cavia Porcellus), male, healthy, local strain, 3-4 months old, 300-350 grams weight, one hybrid, with total of 36 divided into two group (18 each). The control group was given exposure to UVB light and base cream while the treatment group was given exposure to UVB light and black soybean extract cream 30%. The expression of the Tyrosinase enzyme was examined using the immunohistochemical method, whereas the calculation of the amount of melanin was examined using the histopathological method by Masson-Fontana staining. The results showed the mean amount of Tyrosinase in the treatment group was significantly lower than the control group (15.05 ± 2.27% vs 37.36 ± 3.88%; p <0.001). The mean melanin expression in the treatment group was also lower than the control group (4.62 ± 2.27% vs 26.43 ± 3.91%; p <0.001). Based on the results of this study, it was concluded that the administration of black soybean extract cream prevented an increase in Tyrosinase levels and melanin levels in guinea pigs that were exposed to Ultraviolet B.***

Keywords: Black soybean extract, Tyrosinase, Melanin, Ultraviolet B

1. Introduction

Aging is a natural process that occurs in every human being. In this process changes will occur in various parts of the body, both changes that occur in the body such as degeneration of brain cells, or changes that appear from the outside such as changes in skin quality. This aging process is caused by intrinsic and extrinsic factors, where these factors can be different in each individual because they are influenced by genetics, activity, and lifestyle. Currently, along with the development of science, especially in the field of anti-aging medicine, the aging process can be prevented, inhibited, and even the individual's age can be extended and improved the quality of life with anti-aging therapy [1-2].

One sign of aging on the skin is the process of hyperpigmentation. The prevalence of hyperpigmentation is quite high especially in tropical countries like Indonesia because of the high enough sun exposure.

Hyperpigmentation is generally caused by increased melanin production and precipitation. Melanin production is stimulated by the pituitary hormone, melanocyte-stimulating hormone, and is stored in ovoid membrane bound organelles known as melanosomes. In melanosomes, at least three enzymes including tyrosinase, β3,4-dihydroxyphenylalanine (DOPA) chrome tautomerase, and protein related tyrosinase are responsible for melanin synthesis. In in vitro studies, melanin after exposure to UV light can form hydrogen peroxide and superoxide anions which can cause mutations in melanocytes and other cells [3].

The melanogenesis process begins with hydroxylation of L-tyrosine to L-dopa and oxidation of L-dopa to dopakuinone; both of these processes require the activity of the enzyme tyrosinase. Dopakuinone will be spontaneously polymerized to form melanin. The tyrosinase enzyme will work directly when stimulated by UV light. Exposure to ultraviolet A (UVA) with a wavelength of 320-400nm produces intermediate pigmentary darkening where this pigmentation begins 2-3 days after exposure and lasts for 10-14 days [4].

At present many materials have been found to inhibit the occurrence of hyperpigmentation, including having a function as a tyrosinase inhibitor, these materials include: ascorbic acid, arbutin, kojic acid, mercury and hydroquinone [5].

Many studies have been conducted to prove the benefits of black soybean extract as an active ingredient in topical preparations to prevent melanogenesis, one of which is a study conducted by Lai, who in his research in vitro stated

that the sprout extract from black soybean (Glycine max (L) Merrit) proven to inhibit the activity of the tyrosinase enzyme so that it inhibits the melanogenesis cycle but until now there has been no research that proves the effectiveness of in vivo extracts of black soybean (Glycine max (L) Merrit) as an anti-hyperpigmentation agent in guinea pigs skin [6].

2. Material and Methods

2.1 Black Soybean Extract

Soybean (1000gr) which has been sorted is washed and blended then add 96% ethanol solvent in a ratio of 1: 1 (1000 grams of black soybean: 1000 grams of ethanol 96%) after that maceration for 24 hours at 4°C in a dark bottle tightly closed. Then the solution was filtered using Whatman Paper No. 1 and the filtrate was evaporated for 20 minutes. The results of the evaporation in the form of thick extracts are then weighed, labeled and stored in dark colored bottles at 4^0C.

2.2 Black Soybean Extract Cream

Black soybean extract cream formulation 30%: black soybean extract, Sepigel 305 as an emulsifier with a concentration of 3% mixed into water for 5 minutes, then enter 2% lanol, dimethicon 2%, and 0.5% phenoxyethanol, continue mixing until the ingredients in the form of cream, to get 30% concentration of black soybean extract cream needed 30 grams of extract in a total mixture of 100 grams of cream.

2.3 Animals

The experimental animals in this study were male guinea pigs (Cavia porcellus), 3 months old, brown in color with a weight of 300-350 grams with standard dietary feed using HI-GRO 552 and vegetables. Food composition of guinea pigs consists of 17-20% protein, 3-4% fat, 35-40% carbohydrate. For drinking water is used ad libitum. Drinking water is put into bottles that are hung on the walls of the cage (Smith et al., 1988).

Experimental research was posttest only control group design, using 36 male guinea pigs (Caviaporcellus) which were divided into 2 (two) groups, namely the control group was given UVB exposure and placebo cream and the treatment group were given exposure to UVB rays and black soybean extract cream. The control group was given exposure to UVB light and base cream while the treatment group was given exposure to UVB light and black soybean extract cream 30%. Total UVB radiation for 2 weeks was 390 mJ / cm2, treatment of basic cream and black soybean extract cream 30% in each group was given every day, 20 minutes before and 4 hours after UVB radiation.

2.3 Melanin Staining and Quantification

Briefly, after euthanized, the biopsy of skin was immersed into formalin buffer for a night. The skin dehydrated by using grading ethanol, clearing and embedding into paraffin at 60°C. The thickness of cutting was 5 µm and then placed on object glass with poly-lysine. Slides then deparafinized in a series xylene, rehydrated in grading ethanol, stain with Masson Fontana and quantify as described in Miott (2012) [7].

2.4. Immunohistochemistry of Tyrosinase

Antigen retrieval was using Citrate buffer pH 6 boiled with 700-watt microwave for 15 minutes, then cooled in room temperature. Next, the skin washed 2 times in PBS, then peroxidase blocking for 30 minutes, incubation with rabbit anti rat tyrosinase antibody (1:200) for 1 hour followed with labeled polymer-HRP (Dako Envision) for 30 minutes, and then DAB mixture for 10 minutes. Then counterstain with Gill Hematoxilin then mounting with Entellan. Photomicrograph was using 400x magnification by microscope CX-41 (Olympus, Japan) and camera OptilabPro (Miconos, Indonesia). Picture of skin was obtained and analyzed to count the percentage of epidermal cell which expressed tyrosinase. Each sample was captured 3 fields for 400x magnification.

2.4 Statistic

The data obtained then analyzed with independent T-Test by using SPSS 16.0 software.

3. Results

Between Control and Treatment Group, Independent T-test analysis found significant difference in amount of melanin ($p < 0.05$) (Figure 1). The control group had melanin for 26.44% and the treatment group for 4.62%. This study showed that Black soybean extract cream reduced amount of melanin significantly ($p=0.000$). Figure 3 and 4 showed Masson Fontana staining for melanin in Control and Treatment group respectively.

Independent T-test analysis found significant difference in percentage of epidermal cells that expressed Tyrosinase ($p < 0.05$) (Figure 2). The control group expressed Tyrosinase for 37.36% and the treatment group for 15.05%. There was decrease of Tyrosinase expression for 22.31% between control and treatment group. This study showed that Black soybean extract cream reduced expression of Tyrosinase significantly ($p=0.000$). Figure 5 and 6 showed immunohistochemistry of Tyrosinase in Control and Treatment group respectively.

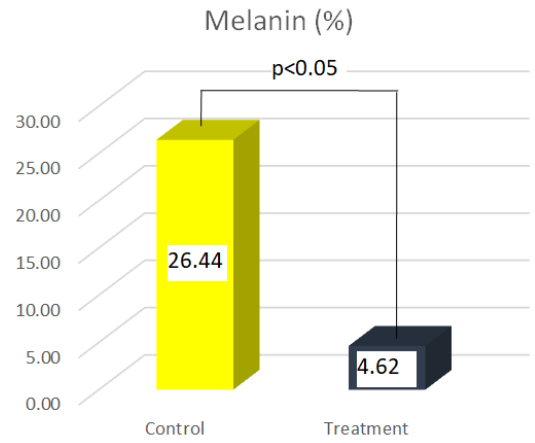

Figure 1: Melanin Quantification between Control and Treatment Group.

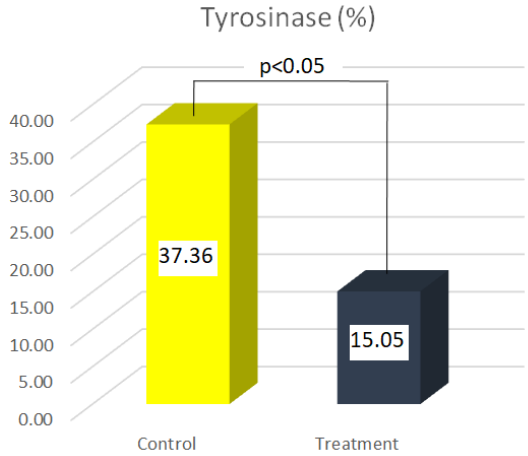

Figure 2: Expression of Tyrosinase Between Control and Treatment Group

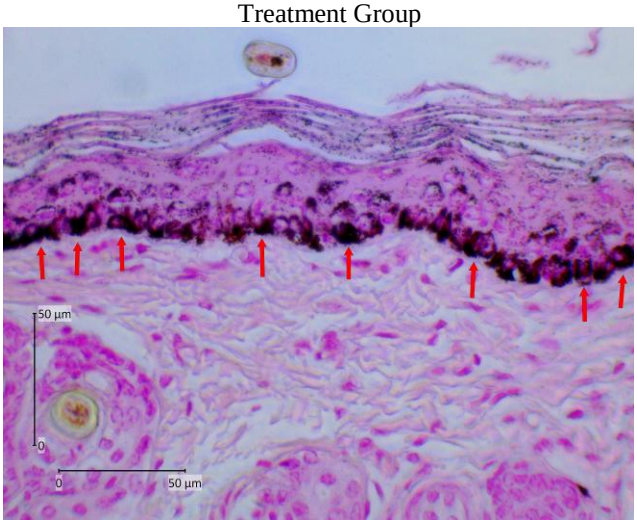

Figure 3: Masson Fontana Staining for Melanin in Control Group (400 X magnification). The amount of melanin (red arrow) was higher than treatment group.

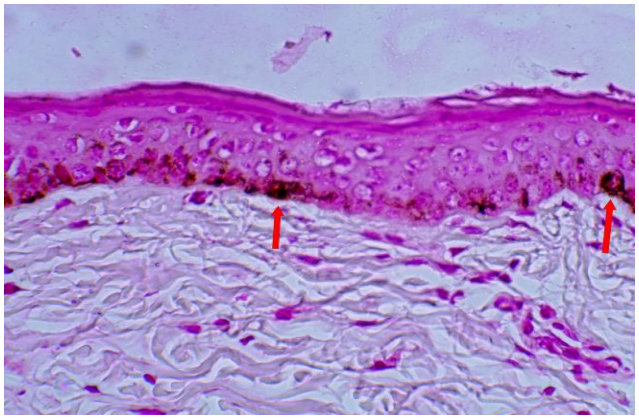

Figure 4: Masson Fontana Staining for Melanin in Treatment Group (400 X magnification). The amount of melanin (red arrow) was lesser than control group.

4. Discussion

Flavanoid is a compound that functions as an antioxidant, to prevent the formation of free radicals that are triggered by exposure to ultraviolet light and also has the ability to inhibit the work of the enzyme tyrosinase which is a key in melanin formation, so as to prevent melanogenesis [8-16]. In this study, the results proved that the expression of tyrosinase in the control group was higher than the treatment group. This shows that the process of tyrosinase formation in the group given 30% black soybean extract cream was inhibited so that the final level was lower than the group given the basic cream ($p < 0.05$). The inhibition of tyrosinase formation in the group given 30% black soybean extract cream in this study also had an impact on the inhibition of melanin formation, because melanin formation requires tyrosinase. The mean of melanin in the control group was more than in the treatment group. Comparative analysis showed that the p value <0.001, which means that the amount of melanin in the 30% black soybean extract cream treatment group was significantly lower than the basic cream treatment group ($p < 0.05$).

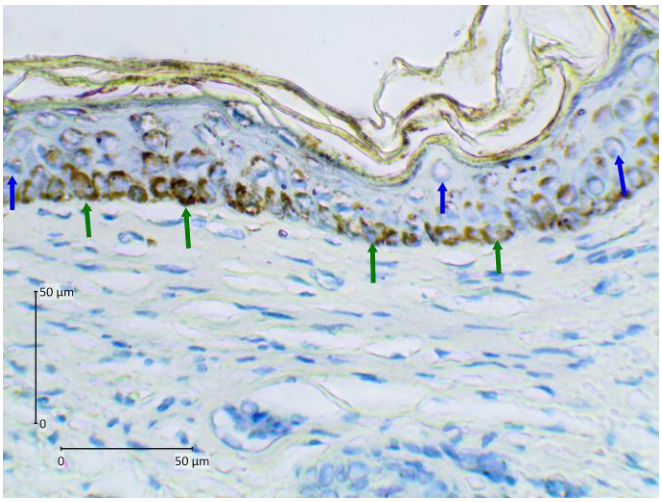

Figure 5: Immunohistochemistry of Tyrosinase in Placebo Group (400X magnification). Abundant of epidermal cells that expressed tyrosinase (green arrow) compared to treatment group. Epidermal cells which were not expressed tyrosinase was blue arrow

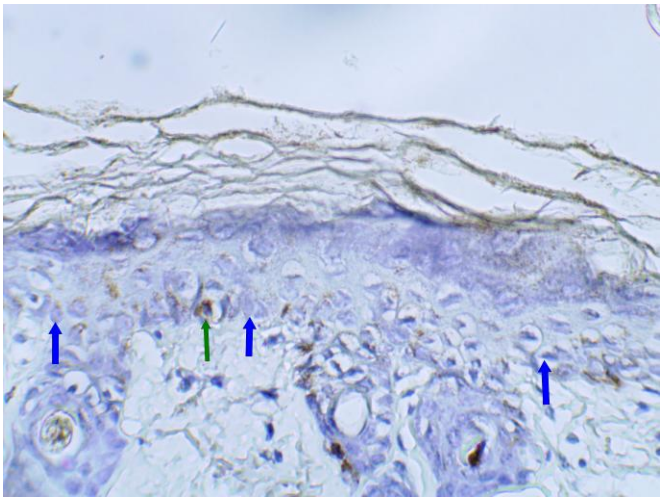

Figure 6: Immunohistochemistry of Tyrosinase in Treatment Group (400X magnification). Less of epidermal cells that expressed tyrosinase (green arrow) compared to control group. Epidermal cells which were not expressed tyrosinase was blue arrow

5. Conclusion

Black soybean extract cream effectively reduced expression of tyrosinase and amount of melanin in epidermal cells of Cavia Porcellus exposed with UVB through its antioxidant properties.

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