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Administration of Moringa (*Moringa oleifera L*) Leaf Ethanol Extract Cream Inhibited the Increase of Tyrosinase Enzyme Levels and Amount of Melanin in Male Guinea Pigs (*Cavia porcellus*) Skin Exposed to Ultraviolet-B

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ABSTRACT

Introduction: The purpose of this study was to prove the effect of administering Moringa leaf ethanol cream extract in inhibiting the increase in tyrosinase level and the amount of melanin in the skin of male guinea pigs (*Cavia porcellus*) exposed to ultraviolet-B (UVB).
Methods: This research was an experimental study using a randomized post-test only control group design method. The research subjects were 30 male guinea pigs divided randomly into five groups: group without cream, group with basic cream, group with 5%, 10% and 20% Moringa leaf ethanol cream extract. The five groups were exposed to UVB lamps for 15 days, and skin samples were taken 48 hours after the last exposure to UVB to examine the amount of melanin using Masson Fontana staining and tyrosinase enzyme using ELISA method.
Results: The best reduction in tyrosinase enzyme was in the group given 20% of Moringa leaf extract cream (10.63 ± 6.53 ng/mL) with a p value of 0.004 ($p<0.05$). Based on the amount of melanin, the highest mean amount of melanin was found in the group without cream (37.40 ± 16.77) and the lowest was in the treatment group given 20% Moringa leaf cream extract (10.63 ± 6.53) with a p value of 0.008 ($p<0.05$).
Conclusion: Administration of Moringa leaf (*Moringa oleifera L.*) ethanol cream extract inhibits the increase in tyrosinase enzyme level and the amount of melanin in the skin of male guinea pigs (*Cavia porcellus*) exposed to UVB with an appropriate concentration for safe use of the cream is between 5% and 10%.

Keywords: Moringa leaf (*Moringa oleifera L.*); tyrosinase enzyme; melanin; guinea pig (*Cavia porcellus*); ultraviolet-B

INTRODUCTION

Exposure to ultraviolet B (UVB) light with a wavelength of 290-320 nm results in delayed pigmentary darkening. This stage causes an increase in the tyrosinase enzyme, melanin production, the number of melanocytes and the spread of melanin to keratinocytes. [1] Prolonged exposure to UV radiation on human skin can cause damage such as skin cancer, sunburn, oxidative stress, and photoaging. [2]
Moringa (*Moringa oleifera L.)* contains quercetin, a flavonoid compound which is a stronger antioxidant than vitamins C and E.[3] Flavonoid compounds have been proven to have a tyrosinase inhibitory effect, where the hydroxyl groups on ring A and ring B inhibit the action of tyrosinase.[4] Several studies have examined the potential of Moringa leaf ethanol extract sunblock cream with

concentrations of 1%, 3% and 5%, with the result that the 5% concentration cream had the highest sun protecting factor (SPF) value of 9.397.[5]

Research on cream from ethanol extract of Moringa leaves as an inhibitor of the tyrosenase enzyme to prevent hyperpigmentation is still limited, therefore it is necessary to carry out further studies regarding the potential of cream from ethanol extract of Moringa leaves by measuring the levels of the tyrosenase enzyme and the amount of melanin. This research aims to prove that administering Moringa leaf ethanol extract cream can inhibit the increase in tyrosinase enzyme levels and the amount of melanin in the skin of male guinea pigs exposed to ultraviolet-b light.

MATERIAL AND METHOD

This study was an experimental post-test only control group design using male guinea pig, aged 10-12 weeks, with body weight of 200-250 grams. The material used in this study was Moringa leaf ethanol cream extract. The sample was divided into five groups: control group (P0), treatment group 1 (P1) which received placebo cream, treatment group 2 (P2) which received 5% Moringa leaf ethanol cream extract, treatment group 3 (P3) 10% Moringa leaf ethanol cream extract, and treatment group 4 (P4) which received 20% Moringa leaf ethanol cream extract. Each group was exposed to UV light three times a week for 15 days. The treatment group was smeared with Moringa leaf ethanol cream extract two times a day. Then the back skin tissue was taken and examined for the amount of melanin by staining Masson Fontana and examined for Tyronase enzym levels using ELISA kit. Data is recorded and analyzed using SPSS version 25 for Windows.

RESULTS

The results of bivariate analysis of tyrosinase enzyme levels between groups showed that the P3 group showed the lowest decrease in tyrosinase enzyme levels, when compared with the control group (10.88 ± 4.37, p = 0.004). Data are presented in table 1.

Table 1. Bivariate Analysis of Tyrosinase enzyme levels on Administration of Moringa Leaf Cream Extract

Group	Tyrosinase enzyme levels + SD (ng/mL)	P-value
P0	21,15±3,32	0.004
P1	19,92±3,38	
P2	12,47±6,13	
P3	10,88±4,37	
P4	13,33±6,52	

In the results of the bivariate analysis on the amount of melanin, the highest mean value was found in the control group which was only exposed to UV light at a dose of 65 mJ/cm, 37.40 ± 16.77. Meanwhile, the lowest mean value for the amount of melanin was found in the treatment group given 20% Moringa leaf extract cream, namely 10.63 ± 6.53. Data are presented in table 2. Histopathological images of each group with Masson-Fontana staining and 100x magnification are presented in Figure 1.

Table 2. Bivariate Analysis of Melanin Amount on Administration of Moringa Leaf Cream Extract

Group	Melanin amount ± SD	P-value
P0	37,40±16,77	0,008
P1	20,80±11,23	
P2	11,20±9,67	
P3	13,76±6,07	
P4	10,63±6,53	

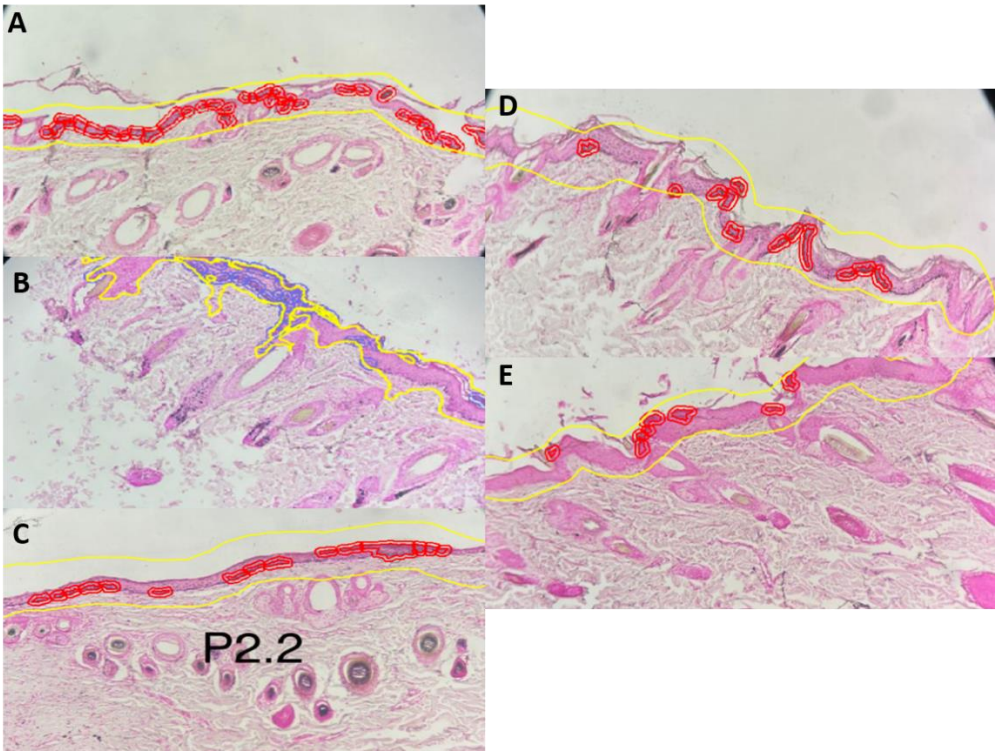

Figure 1. Histopathological images of each group with Masson-Fontana staining and 100x magnification. (A) Group P0, (B) Group P1, (C) Group P2, (D) Group P3, (E) Group P4.

DISCUSSION

In this study, the results of the tyrosinase enzyme were significantly lower in the treatment group that was applied with 10% Moringa leaf ethanol extract cream compared to the other groups, with a comparative analysis of p value <0.05. The 10% concentration has the best level of inhibition of the melanogenesis process. In the treatment group, the results obtained with the lowest mean were significantly 10.88 ± 4.37 ng/L. Meanwhile, in the treatment group with the highest concentration of 20% there was an inhibitory ability so that the mean value increased to 13.33 ± 6.52. This probably occurs because the added concentration affects the ability of antioxidant compounds so that they are not optimal for stabilizing free radicals which cause antioxidant compounds to become prooxidants.[6] In this study, it was also found that the mean amount of melanin decreased significantly in the treatment group that was applied with Moringa leaf ethanol extract cream compared to the group without cream with a comparative analysis of p value <0.05. Inhibition of increasing levels of the tyrosinase enzyme in this study affects the amount of melanin obtained because the melanogenesis process requires the enzyme tyrosinase as a key enzyme. However, in contrast to the results of the average levels of the tyrosinase enzyme, the lowest mean amount of melanin was found in the 20% concentration group with a mean value of 10.63 ± 6.53. This may occur due to other factors that can inhibit the melanogenesis process apart from inhibition of the tyrosinase enzyme, namely inhibition of melanosome transfer.[7]

The antioxidant activity of phenolics is often lost to prooxidants at high concentrations. Apart from that, the influence of the antioxidant structure, sample conditions, the presence of metal ions and the use of extracts with very strong antioxidant activity capabilities also cause prooxidant reactions to occur.[8] Moringa leaf ethanol extract has a strong antioxidant effect with an IC50 concentration of 100 ppm so it can prevent the formation of

Reactive Oxygen Species (ROS) and increase the amount of melanin even at low concentrations. IC50 is the concentration of an inhibitor required to inhibit half of the enzyme activity under tested conditions. An IC50 value below 100 μg/ml indicates the strongest potential in inhibiting the strongest tyrosinase enzyme activity, an IC50 value between 100-450 μg/ml indicates a weak inhibitory ability, and an IC50 value between 450-700 μg/ml indicates a weak inhibitory ability.[9]

The effectiveness of Moringa leaf ethanol extract cream in inhibiting increases in levels of the tyrosinase enzyme and the amount of melanin is due to the content of active compounds such as flavonoids, phenols and tannins which act as antioxidants and competitive inhibitors of the tyrosinase enzyme. Flavonoids as metal ion chelators on the hydroxyl side and/or on the ketone side and phenols as scavenger antioxidants release one hydrogen atom from the hydroxyl group, thus preventing the formation of Reactive Oxygen Species (ROS) and the occurrence of melanogenesis, that is, there is no increase in melanocyte activity to produce melanin, displacement of melanocytes to keratinocytes and tyrosinase enzyme activity.[10] Tannins are phenolic compounds that have a molecular weight of 500-3000 daltons (DA). Tannins can prevent oxidative damage to DNA in two ways, one is by binding to metals, especially iron and the other is by fighting free radicals directly. Tannin also has a role as an inhibitor of the tyrosinase enzyme in the melanin biosynthesis process, so that an increase in melanin production does not occur after exposure to UVB light.[11]

No previous research has evaluated the levels of the tyrosinase enzyme and the amount of melanin after administering Moringa leaf extract in vivo. Research that has been carried out in vivo using another extract, 2.5% cempedak leaf ethanol extract cream which was studied at Udayana University by Ramadhani (2021) contains the same active compounds as Moringa leaf extract cream, namely flavonoids, phenols and tannins which proves its effectiveness in inhibiting the increase in levels of the tyrosinase enzyme and the amount of melanin in the skin of guinea pigs exposed to ultraviolet B light with the result that there was a significant difference in the mean levels of the tyrosinase enzyme and the mean amount of melanin which was lower in the treatment groups 11.60±0.34ng/L and 1, 96±0.67% compared to the control group 30.95±3.43ng/L and 20.54±2.59ng/L. These results are in line with research on Moringa leaves which showed a significant difference in the inhibition of tyrosinase enzyme levels and the amount of melanin in the control group (P0) 21.86 ± 3.32 and 37.40 ± 16.77, there was a decrease in the amount of tyrosinase enzyme in the treatment group with the lowest result was at a concentration of 10% (P3) 10.88 ± 4.37 and the lowest amount of melanin was at a concentration of 20% (P4) 10.63 ± 6.53. This is possibly due to the presence of active compounds of flavonoids, phenols and tannins as antioxidants which inhibit the increase in tyrosinase enzyme levels and the amount of melanin in guinea pig skin exposed to ultraviolet B light.

The antioxidants contained in Moringa leaves are known to reduce black spots on the face and therefore brighten facial skin.[12] However, until now there has been no research showing that Moringa leaf extract has an antihyperpigmentation effect in vivo. This study aims to prove that Moringa leaf ethanol extract cream given topically has an effect in inhibiting the increase in tyrosinase enzyme levels and the amount of melanin with an optimal and safe concentration of 5-10% to avoid the toxic effects of using Moringa leaf ethanol extract cream with higher concentration. It is hoped that the research will benefit from administering 5-10% Moringa leaf ethanol extract cream as an alternative for preventing or treating hyperpigmentation on the skin.

This research has several weaknesses due to the author's limitations, including the selection of Moringa leaves from types and regions and different solvents from previous research which influence the results of the phytochemical test of Moringa leaf extract. In this study, no examination was carried out on the quercetin compound which is known to have a strong antioxidant as an inhibitor of the tyrosinase enzyme due to research limitations, therefore further research needs to be carried out. Other studies also need to be carried out to compare the effectiveness of Moringa leaf ethanol extract with hydroquinone as the gold standard treatment for hyperpigmentation as well as further research conducted on humans before it can be widely used by the public.

CONCLUSION

Based on the research results, it can be concluded that the ethanol extract cream of Moringa leaves can inhibit the increase in tyrosinase enzyme levels % and the amount of melanin in the skin of guinea pigs. However, the safest concentration to use is 5-10% to avoid toxicity effects.

Declaration by Authors

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