The Difference between the Effect of Green Tea Cream and Tocopherol on Decreasing Level of Tyrosinase Enzyme and Amount of Melanin in Rattus norvegicus Exposed to UVB Rays

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ABSTRACT

Introduction: Green tea and tocopherol are potent antioxidants used to treat melasma. However, whether green tea or tocopherol is superior remains unclear. Objective: To compare the effectiveness between green tea and tocopherol in decreasing the amount of melanin and tyrosinase level in wistar rats exposed to UV rays.

Methods: The experimental study took 30 male Wistar rats randomly and divided them into 3 groups. The control group (C-G) was given basic topical cream, GT-G was given green tea topical cream and TC-G was given tocopherol topical cream. All rats were exposed to UVB every Monday, Wednesday and Friday for 4 weeks, whereas topical creams were smeared every day. Topical creams smearing on the same day with UVB exposure was performed 20 minutes before exposure and 4 hours after UVB exposure. The doses of UVB were 50 mJ/cm² in first week, 70 mJ/cm² in the second week and 80 mJ/cm² in the third and fourth weeks. The amount of melanin was measured using pixel method and the tyrosinase level was measured using ELISA.

Results: Anova analysis indicates that the amount of melanin and tyrosinase level are significantly different between groups, p<0.05. Post Hoc LSD analysis indicates that the amount of melanin in GT-G and TC-G are significantly lower than that of C-G, p<0.05. The amount of melanin in GT-G is lower than that of TC-G, p<0.05. The tyrosinase level in GT-G is significantly lower than that of C-G and TC-G, p<0.05. Meanwhile, the tyrosinase level in TC-G is lower than that of C-G but insignificantly, p>0.05.

Conclusion: Green tea topical treatment is significantly capable of decreasing the amount of melanin and tyrosinase level better than tocopherol.

Keywords: Green tea, tocopherol, amount of melanin, tyrosinase level

INTRODUCTION

Melasma is a problem to people living in tropical region, especially women. A woman does not feel confident when she has dull face. Melasma, a cause of dull face, drives women to search for any treatment to get rid of it (Hadiyati PU, Sibero HT, & Apriliana E, 2014; Videira, Moura, & Magina, 2013). Melasma incidents to pregnant women of Hispanic race in Mexico are up to 80%. In Indonesia, its incidents are expected to be at a rate of 0.2 - 4% of those with skin
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METHODS

This experimental research apply a Post Test Only Control Group design. Thirty male wistar rats (Rattus norvegicus), 2 months old, were divided into 3 groups. The control group (C-G) was smeared with basic cream; Green tea group (GT-G) was smeared with green tea cream; Tocopherol group (TC-G) was smeared with tocopherol cream. Before commencement, all rats were acclimatized by group for one week. Before treatment, all rats’ back was shaved, exposed to UVB rays and smears with cream according to their respective group for 0.2 mg/cm² of rat skin surface area. UVB rays exposure was performed to all rats every Monday, Wednesday and Friday for 4 weeks, while the cream was smeared every day. The creams were smeared on the same day with UVB exposure 20 minutes before exposure and 4 hours after exposure. The dose of UVB exposure was 50 mJ/cm² for 50 seconds in the first week, 70 mJ/cm² for 70 seconds in the second week and 80 J/cm² for 80 seconds in the next two weeks, thus the total UVB exposure for 4 weeks was 840mJ/cm² (Miot, Brianezi, Tamega, & Miot, 2012). The rats were left for twenty four hours after the last radiation in order to remove the effect of acute radiation. In the end of research, blood was taken from orbital sinus to

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count the amount of melanin and tyrosinase level. The amount of melanin was measured with pixel method and tyrosinase level was measured with ELISA method. This research was conducted upon approval from the ethics committee of Faculty of Medicine, Unissula no.92/II/2018/Komisi Bioetik.

**Basic Cream Preparation**

Stearic acid 14.5gram was inserted into porcelain dish, put on boiled water and stirred until melted. Borax 125mgwas added and stirred, then tri ethanolamine 1.5ml was inserted and stirred. Further, glycerin 10ml was inserted and stirred, added with distilled water 25ml bit by bit until homogenously mixed and cream base was formed.

**Green Tea Cream Preparation**

Ingredient preparation undergo leaf weathering, cooling, grinding, drying and sorting phases. The unprocessed natural ingredient preparation was then extracted by dissolving it in ethanol 60%. It was then extracted with M AEdaya microwave 450W for 8 minutes. Post Hoc statistical analysis on Melanin and Tyrosinase between group. * p<0.05

| Variable               | Groups          | p-value     |
|------------------------|-----------------|-------------|
|                        | C-G N=10 Mean±SD | GT-G N=10 Mean±SD | TC-G N=10 Mean±SD |               |
| Melanin (%)            | 17.81±1.93      | 5.97±1.59   | 9.82±1.72         | >0.05*        |
|                        |                 |             |                   | >0.05**       |
|                        |                 |             |                   | <0.05***      |
| Tyrosinase (ng/ml)     | 2.23±0.49       | 1.023±0.78  | 2.05±0.71         | >0.05*        |
|                        |                 |             |                   | >0.05**       |
|                        |                 |             |                   | <0.05***      |

Table 1. Data of Mean Amount of Melanin and Tyrosinase Level

**Figure 1. Amount of melanin obtained from Masson-Fontana Dying methods on Skin Epidermis in each group**

**Figure 2. Post Hoc statistical analysis on Melanin and Tyrosinase between group. * p<0.05; ns: not significant**
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Tocopherol Cream Preparation
This research uses lotion of pharmaceutical product from a factory containing tocopherol obtained from pharmacy.

Tyrosinase Level Examination
Ophthalmic vein blood preparation was taken from each rat for 1ml and anti-coagulation EDTA-Na2 was applied. The blood was centrifuged for 15 minutes at the speed of 1000 rotations at 2-8°C. The supernatant produced was immediately tested after the total volume of working solution x number of wells to be used was prepared. The examination method used was ELISA.

Melanin Amount Measurement
Biopsy on rat skin area of 2 x 2cm was made, then immersed into 40% formalin solution. Preparation was made through fixation, dehydration, clearing, embedding and cutting phases, then dying with Masson-Fontana. The dying results are in the form of black melanin granule with pink cell nucleus and pale pink cytoplasm. Counting was then performed using digital analysis method, where each preparation was shot using camera Optilab Pro and microscope Olympus CX21FSI at 400x magnification. The field of view was where melanin exists the most as marked with black area. The counting was performed with the formula: Amount of melanin = (Melanin pixel)/(Epidemis pixel) ×100%.

Data Analysis
The data collected were then analyzed using Shapiro-Wilk test to determine the data distribution. The analysis result shows that the data were normally distributed, p>0.05. Homogeneity test was then conducted with Levene’s test. The analysis result shows that the data were homogenous, p>0.05. Considering that the data is normally distributed and homogenous, the statistical test used is Anova. A One Way Anova test was conducted, followed with Post Hoc LSD test. The analysis result is significant if p<0.05.

RESULTS
After application of topical cream for 4 weeks, results were obtained as presented in table 1. The research results show that the lowest amount of melanin is that of group GT-G, followed with group TC-G and the highest is of the control group C-G (figure 1). Similar with the tyrosinase level, group GT-G has the lowest tyrosinase level, followed with group TC-G, and the highest level is that of control group (C-G). A statistical analysis needs to be conducted to examine whether there is difference in the amount of melanin and tyrosinase level between the groups. Considering that the data is normally distributed and homogenous, the statistical test used is Anova. The result of Anova shows that there is significant difference between the amount of melanin and tyrosinase level of the three groups, p<0.05. Further, a Post Hoc LSD test needs to be conducted to examine which group is significantly different, as explained below.

Amount of Melanin
The amount of melanin of groups GT-G and TC-G is significantly lower than that of group C-G, p<0.05. The amount of melanin of group GT-G is significantly lower than that of group TC-G, p<0.05 (figure 2). This describes that green tea’s effect of reducing melanin amount is better than that of tocopherol.

Tyrosinase Level
The level of tyrosinase of group GT-G is significantly lower than that of groups C-G and TC-G, p<0.05. The level of tyrosinase of group TC-G is insignificantly lower than that of group C-G, p>0.05 (figure 2). This describes that green tea’s effect of reducing tyrosinase enzyme level is better than that of tocopherol.

DISCUSSION
This research result shows that green tea cream application may reduce melanin amount and tyrosinase level. Meanwhile, tocopherol cream, even if it may reduce melanin amount, but cannot reduce tyrosinase level. This describes that the effect of green tea cream application is better than tocopherol cream application. This research result is in line with the invitro study conducted by Kim CY, et al. on melanin cell A, stating that green tea application evidently reduces tyrosinase enzyme activity (Young Chul Kim et al., 2015). Other invitro study reported by Dissanayake CY, et al. also states that green tea application to B16-F10 melanoma cell evidently inhibits melanin synthesis through reduction of gene expression responsible for melanin synthesis (Dissanayake, Moon, Yang, Lee, & Han, 2018). On the other hand, tocopherol cream application, even if it may reduce melanin amount, but it cannot reduce tyrosinase level significantly. This research result is slightly different from the invitro study on B16 melanoma cell, stating that various analog tocopherols evidently reduce melanin amount and
tyrosinase activity significantly (Albakry, Amiyati, & Hasan, 2018). Among the 8 analog tocopherols, that ones with high potential are D-β-tocopherol and D-γ tocopherol (Suárez-Jiménez et al., 2016). In this study, the analog tocopherol used is D-α.

Tocopherol cream, even if there is a reduction of tyrosinase level, but is insignificant compared to the control. Other study conducted by Keen & Hassan (2016) also shows that α-tocopherol only has minimal depigmentation effect on melasma, through lipid peroxidation on melanocyte membrane, intracellular glutathione binding and tyrosinase inhibition (Keen & Hassan, 2016).

The insignificant reduction of tyrosinase level is expectedly also related to gene polymorphism. The study conducted by Tripathi et al. states that general non-pathologic polymorphism is identified in tyrosinase gene of Caucasian race, with codon 402 in the form of CGA (arginine) or CAA (glutamine) (Tripathi, Giebel, Strunk, & Spritz, 2018). In addition, melanin synthesis is also influenced by tyrosinase related Protein 1 (TYRP1) and Dopachrome T automerase (DCT). The three enzymes work together and synthesize two types of melanin, namely eumelanin and pheomelanin (Videira et al., 2013). Polymorphism also occurs to black American people, but codon 402 CAA is not detected in Oriental population. Tyrosinase activity with codon 402 Gln is also influenced by temperature, in which activity is reduced 75% at 37°C compared to activity at 31oC, while the enzymatic activity of codon 402 Arg is not influenced by temperature (Cichorek, Wachulska, Stasiewicz, & Tyminska, 2013; D’Orazio, Jarrett, Amaro-Ortiz, & Scott, 2013).

The reduction of melanin amount because of green tea application is caused by flavonoid’s antioxidant nature and able to inhibit tyrosinase enzyme activity (Woolery-Lloyd & Kammer, 2011; Young Chul Kim et al., 2015). The most important flavonoid existing in green tea is Epigallocatechin Gallat (EGCG). EGCG is able to inhibit tyrosinase activity and prevent tyrosinase formation. Thus, leading to melanin synthesis inhibition. EGCG as natural antioxidant will catch free radicals arising from UVB rays exposure producing Reactive Oxygen Species (ROS) (Peluso & Serafini, 2017). ROS will influence mRNA tyrosinase regulation, triggering formation of tyrosinase, which catalyzes the main reaction of melanogenesis by hydroxylation L-tyrosine to L-dopa, then oxidizing L-dopa to dopaquinone. Melanin synthesis produces eumelanin after passing through indol quinon pathway or become pheomelanin after passing through cysteiny1 DOPA pathway (D’Orazio et al., 2013).

This melanin amount reduction conforms to previous studies that green tea’s polyphenol serves as photoprotective agent and able to prevent skin pigmentation due to UVB rays exposure. This depigmentation occurs since green tea contains abundant catechin in the form of natural antioxidant (Oliveira, 2012). The research conducted by Chasissa M, et al. (2016) also states that flavonoid is natural polyphenol able to depigment skin by directly inhibiting tyrosinase activity (Charissa, Djadisasastra, & Elya, 2016). In addition, tannin is strong antioxidant and able to inhibit tyrosinase (Chai et al., 2014). Further, various studies also state that antioxidant may suppress oxidant’s negative effect, bind free radicals and inhibit melanin synthesis (Nichols & Katiyar, 2011).

On the other hand, some studies also state that tocopherol may directly inhibit tyrosinase activity in melanogenesis mechanism(Suárez-Jiménez et al., 2016) and even reduce up to 70% pigmentation and reduce 40% tyrosinase activity compared to the control group (Keen & Hassan, 2016). Therefore, we may state that green tea application may inhibit melanin formation. Similarly, tocopherol may inhibit melanin formation, but in this research, green tea does it more significantly.

This study’s limitation is that it does not examine the level of antioxidant, pharmacokinetics and pharmacodynamics of green tea or tocopherol influencing melanin amount and tyrosinase level.

CONCLUSION

The research results show that green tea cream and tocopherol cream applications are able to reduce tyrosinase activity and melanin synthesis. However, green tea’s capability to reduce melanin amount and tyrosinase level is better than tocopherol.

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CONFLICT OF INTEREST

There is no conflict of interest in this publication.

REFERENCES

Albakry, N. A., Amiyati, Y., & Hasan, Z. A. A.
(2018). The Mechanism and Potential of Palm Vitamin E as a Skin-lightening Agent. Palm Oil Developments 68, 6, 9–13.

Ayala, A., Muñoz, M. F., & Argüelles, S. (2014). Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal Oxidative Medicine and Cellular Longevity, 2014, 31. https://doi.org/10.1155/2014/360438

Chai, W.-M., Yan, C.-L., Chen, X.-X., Gao, Y.-S., Tian, L., Feng, H.-L., … Shi, Y. (2014). Isolation and Purification of Condensed Tannins from Flamboyant Tree and Their Antioxidant and Antityrosinase Activities. Applied Biochemistry and Biotechnology, 173(1), 179–192. https://doi.org/10.1007/s12010-014-0828-z

Charissa, M., Djajadisastra, J., & Elya, B. (2016). Uji Aktivitas Antiksidan dan Penghambatan Tirosinase serta Uji Manfaat Gel Ekstrak Kulit Batang Taya (Naulica subdita) terhadap Kulit. Jurnal Kefarmasian Indonesia, 6(2), 98–107.

Cichorek, M., Wachulksa, M., Stasiewicz, A., & Tyminska, A. (2013). Skin melanocytes: Biology and development. Postepy Dermatologii i Alergologii, 30(1), 30–41. https://doi.org/10.5114/pdia.2013.33376

D’Orazio, J., Jarrett, S., Amaro-Ortiz, A., & Scott, T. (2013). UV radiation and the skin. International Journal of Molecular Sciences, 14(6), 12222–12248. https://doi.org/10.3390/ijms140612222

Deny, F., & Hakim, Z. (2006). Penggunaan Vitamin E Dan Vitamin C Topikal. Majalah Kedokteran Andalas, Padang, (2) 30(2).

Dissanayake, C. Y., Moon, H. H., Yang, K. M., Lee, Y., & Han, C. H. (2018). The effects of green tea (Camellia sinensis) flower extract on melanin synthesis in B16-F10 melanoma cells. Korean Journal of Veterinary Research, 58(2), 66–72. https://doi.org/10.14405/kjvr.2018.58.2.65

Hadiyati PU, Sibero HT, & Apriliana E. (2014). Kualitas Hidup pada Pasien Melasma di RSUD Dr. H. Abdul Moeloek Lampung. Jurnal Kedokteran Universitas Lampung, 3(5), 130–138.

Hussain, T., Tan, B., Yin, Y., Blachier, F., Tossou, M. C. B., & Rahu, N. (2016). Oxidative Stress and Inflammation: What Polyphenols Can Do for Us? Oxidative Medicine and Cellular Longevity, 2016, 1–9. https://doi.org/10.1155/2016/7432797

Jusuf, N. K. (2017). Pattern of pigmentation disorder in Cosmetic Dermatology Clinic H. Adam Malik General Hospital, Medan, 2012 -2015. J Gen Proced Dermatol Vereenol Indones, 2(1), 1–6.

Keen, M., & Hassan, I. (2016). Vitamin E in dermatology. Indian Dermatology Online Journal, 7(4), 311. https://doi.org/10.4103/2229-5178.185494

Lim, J. Y., Kim, O. K., Lee, J., Lee, M. J., Kang, N., & Hwang, J. K. (2014). Protective effect of the standardized green tea seed extract on UVB-induced skin photoaging in hairless mice. Nutrition Research and Practice, 8(4), 398–403. https://doi.org/10.4162/nrp.2014.8.4.398

Miot, H. A., Brianzei, G., Tamega, A. de A., & Miot, L. D. B. (2012). Techniques of digital image analysis for histological quantification of melanin. Anais Brasileiros de Dermatologia, 87(4), 608–611. https://doi.org/10.1590/s0365-05962012000400014

Nichols, J. A., & Katiyar, S. K. (2011). Polyphenols: skin photoprotection and inhibition of photocarcinogenesis. Mini Reviews in Medicinal Chemistry, 11(14), 1200–1215. https://doi.org/10.1007/s00403-009-1001-3.Skin

Oliveira, R. M. M. De. (2012). Quantification of catechins and caffeine from green tea (Camellia sinensis) infusions, extract, and ready-to-drink beverages. Food Science and Technology (Campinas), 32(1), 163–166. https://doi.org/10.1590/S0101-20612012000500009

Peluso, I., & Serafini, M. (2017). Antioxidants from black and green tea: from dietary modulation of oxidative stress to pharmacological mechanisms. British Journal of Pharmacology, 174(11), 1195–1208. https://doi.org/10.1111/bph.13649

Prasanth, M. I., Sivamaruthi, B. S., Chaiyasut, C., & Tencomnao, T. (2019). A review of the role of green tea (camellia sinensis) in antiphotoaging, stress resistance, neuroprotection, and autophagy. Nutrients, 11(2). https://doi.org/10.3390/nu11020474

Suárez-Jiménez, G. M., López-Saiz, C. M., Ramírez-Guerra, H. E., Ezquerra-Brauer, J. M., Ruiz-Cruz, S., & Torres-Arreola, W. (2016). Role of endogenous and exogenous tocopherols in the lipid stability of marine oil systems: A review. International Journal of Molecular Sciences, 17(12). https://doi.org/10.3390/ijms17121968
Tripathi, R. K., Giebel, L. B., Strunk, K. M., & Spritz, R. A. (2018). C E N E EXPRESSION is associated with temperature-sensitive enzymatic activity. Cichago Medical School, 1(2), 103–110.

Videira, I. F. dos S., Moura, D. F. L., & Magina, S. (2013). Mechanisms regulating melanogenesis. An Bras Dermatol, 88(1), 76–83.

Woolery-Lloyd, H., & Kammer, J. N. (2011). Treatment of Hyperpigmentation. Seminars in Cutaneous Medicine and Surgery, 30(3), 171–175. https://doi.org/10.1016/j.sder.2011.06.004

Young Chul Kim, Choi, S. Y., & Park, E. Y. (2015). Anti-melanogenic effects of black, green, and white tea extracts on immortalized melanocytes. Journal of Veterinary Science, 16(2), 135–143. https://doi.org/10.4142/jvs.2015.16.2.135