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RESEARCH ARTICLE

Effects of Topical Tomato (Lycopersicon Lycopersicum L) Extract on Malondyaldehyde Level and the Number of Melanin in Skin Caused by Ultraviolet-B Radiation

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ABSTRACT

Introduction: *UVB radiation on skin may increase reactive oxygen species (ROS) level and cause hyperpigmentation. Polyphenol, an antioxidant contained in tomato, is able to quench ROS and inhibit tyrosinase activity.* **Objective:** *This study aims to evaluate the effect of topical tomato lotion on Malondyaldehide (MDA) level and the amount of melanin in skin following UVB radiation.*

Methods: *In this experimental study, 28 female Balb/c mice are randomly divided into 4 groups: control group (C-G) are given with base lotion, and Lycopersicum groups 0.14 (L-014), 0.7 (L-07) and 1.4 (L-14) are given with tomato lotion of concentration of 0.14%, 0.7% and 1.4% daily, respectively. All of the mice are exposed to UVB at 1MED once every two days for two weeks before lotion application. On day 16, all mice are terminated, and their skin tissues are prepared for MDA level and the amount of melanin assessment.*

Results: *The ANOVA statistical analysis shows a significant difference in the MDA level and the number of melanin of the groups, ($p<0.05$). The post-hoc LSD analysis shows that the level of MDA in L-14 (1.13) is significantly lower than that in L-07 (2.21), L-014 (2.47), and C-G (5.61), $p<0.005$. The amount of melanin in L-14 (10.00) is significantly lower than that in L-014 (23.50) and C-G (33.67), $p<0.05$. The amount of melanin in L-07 (13.00) is not significantly higher than that in L-14, $p>0.05$. The correlation between MDA level and the amount of melanin shows a strongly positive correlation value ($r2$) = 0.648*

Conclusion: *Topical administration of tomato extract lotion significantly decreases the MDA level and the number of melanin in the mice's skin following UVB radiation.*

Keywords: *UVB, tomato extract, MDA, melanin*

ABSTRAK

Pendahuluan: Paparan UVB pada kulit meningkatkan kadar radikal bebas dan menyebabkan hiperpigmentasi. Plifenol adalah antioksidan yang terkandung dalam buah tomat terbukti mampu menetralkan radikal bebas dan menghambat aktivitas enzim tirosinase.

Tujuan: Penelitian ini bertujuan untuk mengetahui pengaruh ekstrak tomat topikal terhadap kadar Malondyaldehide (MDA) dan jumlah melanin pada kulit yang dipapar UVB.

Metode: Penelitian eksperimental menggunakan 28 ekor mencit BALB/c betina yang dibagi secara acak menjadi 4 kelompok: Kelompok control (C-G) hanya mendapatkan basis lotion, kelompok Lycopersicon 0.14 (L-014), 0.7 (L-07), dan 1.7 (L-17) masing-masing diberi lotion ekstrak tomat 0,14%; 0,7% dan 1,4%. Aplikasi lotion dilakukan sekali sehari sebelum paparan UVB satu MED yang diberikan setiap 2 hari sekali selama 2 minggu. Hari ke-16 mencit diterminasi, kulit punggung diambil untuk pemeriksaan kadar MDA dan jumlah melanin jaringan. Analisis statistik yang digunakan adalah *one way* ANOVA, *post-hoc* LSD, dan korelasi Pearson.

Hasil: Uji ANOVA menunjukkan bahwa terdapat perbedaan kadar MDA dan jumlah melanin yang bermakna di antara kelompok, $p<0,05$. Uji *post-hoc* LSD menunjukkan kadar MDA pada L-17 (1,13) lebih rendah bermakna dibanding L-07 (2,21), L-014 (2,47) dan C-G (5,61), $p < 0,005$. Sedangkan jumlah melanin pada L-17 (10,00) lebih rendah bermakna dibandingkan L-07 (23,50) dan C-G (33,67), $p<0.005$. Jumlah melanin pada L-07 adalah 13,00; tidak berbeda bermakna dengan L-17, $p=0,37$. Korelasi kedua variabel kuat dengan nilai korelasi Pearson ($r2$) = 0,648.

Kesimpulan: Pemberian ekstrak tomat topikal menurunkan kadar MDA dan jumlah melanin pada kulit mencit yang terpapar sinar UVB.

Kata kunci: UVB, ekstrak tomat, MDA, melanin

INTRODUCTION

Many people of the equatorial area are exposed to ultraviolet B (UVB) rays at high frequency and intensity, thus they frequently experience severe photoaging reactions. The clinical signs which may occur on skin include wrinkles, hyperpigmentation, melasma,

rough skin, dry skin, sagging skin, severe atrophy, telangiectasias, elastosis, actinic purpura, sunburn, melanoma, precancer lesions, and skin cancer (Pandel et al., 2013). Melasma is more common in people with brown or black skin like Asian, Middle Eastern, Indian, South American people (Wolff, 2009). A research in the United States finds that facial melasma ranks the 5th (8.2%) out of total 1,000 visits of Latin race to clinic (Taylor et al., 2008). Melasma has significantly negative impact on the quality of life since it affects appearance, social life, welfare, emotion, and recreational activity (Jiang et al., 2018).

Nowadays, more and more male and female Asians desire brighter skin. Whitening creams with hydroquinone content are preferred by the society since they are unaware of the increasing risk of sensitivity to ultraviolet rays resulted from damage to melanin pigment producing melanocyte cells. Reduction of the amount of melanin pigment which serves to protect skin makes skin tissue more prone to damage caused by exposure to UVB rays. Tomato fruit is proven to reduce reactive oxygen species (ROS) content and in in vitro research may inhibit tyrosinase enzyme activity which plays a role in melanin pigment synthesis (Zaveri and Patel, 2012). However, it is unknown whether tomato extract administration in the form of lotion will reduce ROS as marked with reduction of malondyhaldehyde (MDA) content and skin brightness as marked with reduction of the amount of melanin induced by UVB rays.

UVB rays induce production of ROS, including hydrogen peroxide, hydroxyl radical, singlet oxygen and superoxide through activation of NADPH oxidase and cyclooxygenase enzymes. The polyphenol compound contained in tomato fruit serves to be the inhibitor of NADPH oxidase (Maraldi, 2013). Lycopene as the main antioxidant contained in tomato fruit besides vitamin C, vitamin E and β carotene is able to reduce ROS content. This reduction of ROS content occurs through 3 mechanism, which are electron transfer, hydrogen abstraction and ROS binding (Kong et al., 2010). Without antioxidant presence, ROS will take hydrogen from methylene group in unsaturated fatty acid which composes cell membrane to form new radicals and cause chain reactions which damage cell membrane (Yin et al., 2011). Free radicals and inflammation process caused by exposure to UVB will trigger melanogenesis process, increasing the amount of melanin. The increasing amount of melanin will protect keratinocyte cells from UVB radiation, but on the other hand causes skin hyperpigmentation. Tomato lotion administration is expected to reduce the amount of melanin through reduction of ROS and tyrosinase enzyme activity because of exposure to UVB (Zaveri and Patel, 2012).

The results of research reported by Wahyono show that tomato (Lycopersicon Lycopersicum L) juice administration per oral is proven to fix oxidative stress marked with MDA level reduction, Activator Protein-1 expression and collagen type 1 increase in mouse's skin because of exposure to UVB rays (Wahyono et al., 2011). Another research also proves that topical administration of tomato extract with 0.14% dose is proven useful as an anti-inflammation in female Balb/c mouse's back. An in vitro study also shows that tomato extract at concentration of 100μg/ml is able to inhibit tyrosinase enzyme activity which plays a role in melanin synthesis with IC50 of 4.08μg/ml (Zaveri and Patel, 2012).

The purpose of this research is to evaluate the effect of tomato extract lotion administration on MDA content and the amount of melanin in mice exposed to UVB rays.

METHODS

This experimental research employs a post-test only control group design and 28 female BALB/c mice (6-8 weeks old, body weight 18-35 gram) randomly divided into 4 groups: Control group (C-G) is only give with base lotion, Lycopersicon groups 0.14 (L-014), 0.7 (L-07) and 1.4 (L-14) are given with tomato extract lotion 0.14%; 0.7% and 1.4% daily, respectively. All groups are exposed to UVB 1 MED before lotion application once every 2 days for 2 weeks. The mice are terminated on day 16 and their back skin tissues are taken for examination of MDA content and the amount of melanin. This research is conducted at the Laboratory of Chemicals of the Faculty of Medicine, Sultan Agung Islamic University, Semarang, the Laboratory of Pathology and Anatomy of Sultan Agung Islamic Hospital, Semarang and the Laboratory of Food and Nutrition of Pusat Antar Universitas (PAU) Gajah Mada University, Yogyakarta. This research is conducted under approval of the Ethics Committee of the Faculty of Medicine, Unissula.

Tomato Extract Lotion Making

Tomatoes are crushed with a blender, weighed for 100g, and macerated using ethanol 96% (1:10). After 1 day, the yield is filtered using glass funnel layered with filter paper until filtrate and residue are produced. The residue is disposed of and the filtrate is evaporated using an evaporator until thick tomato ethanol extract sample is produced. 0.14g of thick tomato ethanol

extract is put into a mortar, added with 96.87ml of ethanol 70% and stirred until they are homogeneous. 2.99ml of glycerol is then added into it and stirred until it is evenly distributed, and then put into a bottle. The formula above is to make a 100ml lotion with 0.14% content. The lotions are made with 0.14%, 0.7% and 1.4% contents.

Exposure to UVB Rays

The fur on the back of all mice is shaved. UVB rays radiation is performed at 40 cm for 8 minutes. The exposure is performed once every 2 days for 14 days. On day 16, all mice are terminated.

MDA Level Measurement

On day 16, all mice are terminated and their back tissues are taken. The blood is disposed of with in situ perfusion using isotonic saline fluid. The tissue is weighed and prepared in 20 mM phosphate buffer at pH 7.4, added with 10 µL 0.5 M BHT in acetonitrile into 1 mL tissue. A centrifugation at 3000x g is conducted at 4°C for 10 minutes. The quantity factor (aliquot) is disposed of, and MDA content is measured immediately with "The NWLSSTM Malondialdehyde Assay (NWK-MDA01)" technique. 10µL of BHT Reagent is added into a microcentrifuge tube. 250µL of calibrator or blood serum sample, 250µL of acid reagent and 250µL TBA reagent are then added consecutively into the tube, and stirred strongly (5 times). The next phase is 60 minutes of incubation at 60°C, and Centrifuge 10,000x g for 2-3 minutes. The material is then moved into a cuvette and the spectrum with 400-700 nm wavelengths is recorded.

Number of Melanin Counting

Mouse's back skin tissue is soaked in 10% buffered formalin solution for 24 hours. The part of tissue to be taken is trimmed. The tissue is then soaked in alcohol at multilevel concentration respectively 30%, 40%, 50%, 70%, 80%, 90% and 96%, each 3 times for 25 minutes. The tissue is then soaked in alcohol clearing agent: xylene 1:1 for 30 minutes and dipped into pure xylene solution until it becomes transparent. Infiltration is conducted four times with pure paraffin, in which the tissue is then soaked in liquid paraffin, and left for ± 24 hours until it becomes solid. It is then serially cut using microtome Leica 820 in 5µ of thickness, in which slices 5, 10 and 15 are taken and stuck onto object glass smeared with adhesive, and stained using Masson Fontana reagent. The slide of tissue is soaked in xylene 2 times, each for 5 minutes, soaked in ethanol 100%, 95% and 70% and dH2O, each for 2 minutes, soaked in Silver Nitrate Fontana solution for 2 hours, incubated at 56ºC in oven, washed using dH2O 3 times, dripped with Gold Chloride 1% solution, left for 5 minutes, washed using dH2O, dripped with Sodium thiosulfate 5% solution, left for 1 minute, washed using dH2O, stained using Nuclear Fast Red for 5 minutes, washed using dH2O 2 times and dehydrated using ethanol 70%, 95% and 100%, each for 20 seconds. Further phase is clearing using xylene 2 times, each for 2 minutes and mounting in xylene based medium. The amount of melanin is manually counted by counting the number of cells in epidermis layer of which cytoplasm is stained black using microscope with 400 times magnification at 3 fields of vision.

Statistical Analysis

The statistical analysis is conducted with Shapiro Wilk and Lavene tests, each to find out the data normality and homogeneity, followed with One Way Anova, Post Hoc and Pearson Correlation tests. The analysis results are considered significant if the p value < 0.05.

RESULTS

The two weeks of research result in the mean of MDA level and number of melanin as presented in table 1.

The lowest mean of MDA content is that of L-14, followed by that of L-07 and of L-014, while the highest MDA level is that of C-G. Likewise, the lowest mean of melanin number is that of L-14, followed by that of L-07 and of L-014, while the highest mean of melanin number is that of C-G. A statistical test is required to find out whether there is significant difference in MDA content and melanin amount between the groups. Considering that Shapiro Wilk and Levene tests show normally and homogenously distributed data ($p>0.05$), the statistical test shall be the One Way Anova. The results of Anova show significant difference between the groups, $p<0.05$. The post-hoc LSD test below is conducted to find out which groups are significantly different.

MDA Content

The results of Post-Hoc LSD test shows that the MDA content of L-014, L-07, and L-14 is significantly lower than that of C-G, $p<0.01$, and the MDA content of L-07 and L-14 is lower than that of L-014, $p<0.05$. Therefore, we may conclude that there is significant difference between all of the groups. Meanwhile, the MDA content of L-14 is significantly lower than that of L-07, $p<0.05$ (figure 1).

Table 1. Mean of MDA level and Number of Melanin of each Group

VARIABLE	Groups				p value
	C-G	L-014	L- 07	L- 14	
	Mean (±SD) n=6	Mean (±SD) n=6	Mean (±SD) n=6	Mean (±SD) n=6	
MDA Level (nmol/g)	5.61 (±0.08)	2.47 (±0.17)	2.21 (±0.23)	1.13 (±0.13)	>0.05* 0.20** 0.00***
Number of Melanin (Σ)	33.67 (±9.13)	23.50 (±4.65)	13.00 (±1.60)	10.00 (±5.21)	>0.05* 0.52** 0.00***

Note: *Shapiro-Wilk **Lavene test ***One way ANOVA

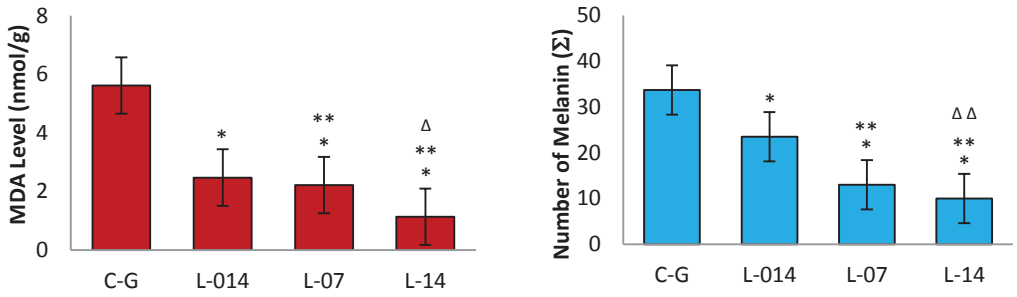

Figure 1. Post Hoc Analysis on MDA level and Number of Melanin. * p<0.05 C-G as Control; ** p<0.05 L-014 as Control; Δ p<0.05 L-07 as control; ΔΔ p>0.05 L-07 as control

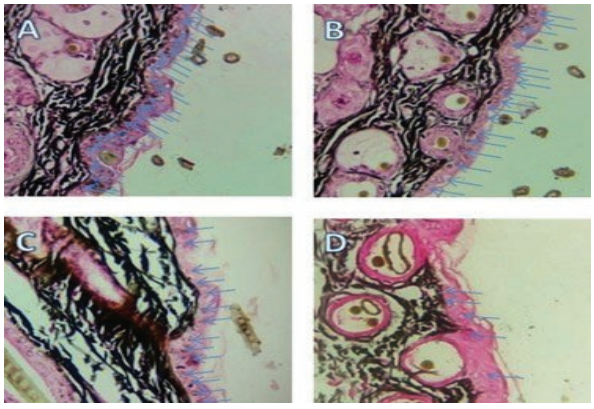

Figure 2. Amount of Melanin in Each Group. A: C-G; B: L-014; C: L-07; D: L-14.

Melanin Number

The results of Post-Hoc LSD analysis show that the amount of melanin of L-014, L-07 and L-14 is significantly lower than that of C-G, p<0.05. The amount of melanin of L-07 and L-14 is significantly lower than that of L-014, p<0.05. Meanwhile, the amount of melanin of L-14 is not significantly different than that of L-07, p>0.05 (figure 1 & 2).

DISCUSSION

The results of this research show that topical tomato extract is able to reduce the MDA content and the amount of melanin of mouse's skin exposed to UVB rays. This conforms to some previous researches. Poncojari Wahyono et al. find that tomato extract administered per oral may reduce the MDA content of mouse's skin tissue exposed to UVB rays (Wahyono et al., 2011). Maitreyi Zaveri et al. in in vitro research also find that tomato extract is able to inhibit tyrosinase enzyme activity with IC50 value of 4.08µg/ml stronger than vitamin C with IC50 value of 6.4µg/ml and goji berry extract with IC50 value of 5.53µg/ml (Zaveri

and Patel, 2012). Oxidative stress fixing effect marked with reduction of MDA and tyrosinase enzyme activity reduction marked with reduction of the amount of melanin are caused by various compounds contained in tomato extract.

Evidence shows that tomato contains various important components, such as lycopene, vitamin C, Vitamin E, β carotene, solanine, saponin, folic acid, malic acid, citric acid, protein, fat, sugar (fructose, glucose), adenine, trigonelin, choline, tomatin, mineral (Ca, Mg, P, K, Na, Fe, Cu, Se, Zn, S and Cl), histamine, and various polyphenols like Naringenin chalcone, Rutin, Quercetin, Chlorogenic acid, Caffeic Acid, Naringenin, Kaempferol-3-rutinoside, Coumaric acid, Ferulic acid, Kaempferol, Myricetin, Cyanidin, Pelargonidin and Delphinidin (Slimestad et al., 2008).

Lycopene is the most effective antioxidant of carotenoids to suppress singlet oxygen radicals much formed in photooxidation. Besides, lycopene has an anti-inflammation effect by inhibiting iNOS pro-inflammatory mediator enzyme produced in macrophage cells after induction by ROS (Ferreira et al., 2013). Lycopene's anti-inflammatory effect is also caused by lycopene's capability to inhibit TNF α and NF-κB and IL-1β (Cha et al., 2017; Lee et al., 2011). β carotene may serve as an anti-inflammation by suppressing NF-kB activity (Cho et al., 2018). Vitamin C may inhibit inflammation process by suppress pro-inflammatory cytokines, which are iNOS and NF-κB (Yazdani Shaik BD and Conti, 2016) while vitamin E suppresses inflammation process by inhibiting cytokines of TNF α and iNOS (Cho et al., 2018). Poncojari Wahyono et al. in a research on mouse induced with CCl4 find that tomato extract consumption of 7g/kg of body weight, 11g/kg of body weight and 15g/kg of body weight may reduce SGOT, SGPT and MDA of liver tissue (Wahyono, 2008). Poncojari Wahyono et al. in another research report that tomato (Lycopersicum pyriforme) juice with dose of 11g/kg of body weight per oral is able to reduce MDA content, AP-1 and increase collagen type 1, and the effect of juice on treatment groups are equal to the group with a combination of vitamin C, lycopene and β carotene (Wahyono et al., 2011).

ROS formation occurs within less than 30 minutes after exposure to UVB rays. Chromophore molecules excited by ultraviolet ray energy may form singlet oxygen radicals in case of reaction with oxygen (Sano et al., 2014). Singlet oxygen radicals may oxidize water molecules to form hydrogen peroxide radical (Khorobrykh et al., 2015). Singlet oxygen radicals may be well suppressed by lycopene and β carotene contained in tomato. Hydrogen peroxide may form other ROS rapidly, like hydroxyl radical. Ceratinocyte cells show NADPH oxidase (NOX) activity which catalyzes and reduces oxygen molecules to superoxide anions (Nauseef, 2014). The study conducted by Weyemi et al. also shows that polyphenol, lycopene and folic acid contained in tomato is able to inhibit NADPH oxidase activity (Weyemi et al, 2012). ROS will then oxidize cell components of DNA, protein, and cell membrane. One final product of cell membrane oxidation process or known as lipid peroxidation is malondyaldehide (MDA). Lycopene is able to provide its hydrogen atom to suppress free radicals and thus prevent initiation and propagation of lipid peroxidation process.

Skin tissue will protect itself from exposure to UVB by forming melanin pigment. Lycopene contained in tomato is able to prevent destruction of cell membrane and lymphocyte cell death because of Nitric Oxide radical attack twice more efficient than β-carotene (Cha et al., 2017). Nitric Oxide is the second messenger serving in melanin pigment synthesis (Ferreira et al., 2013). Polyphenol and antioxidant contained in tomato are able to inhibit melanin pigment formation because of their ability to serve as substrate for tyrosinase enzyme. Polyphenol and antioxidant will be oxidized first ahead of dopa and dopaquinon, thus melanin production will be reduced (Chang, 2009). Meanwhile, vitamin C contained in tomato is able to serve as copper chelator to bind copper atom located at active site of tyrosinase enzyme (Alam et al., 2009). The in vitro research on various plants' extracts conducted by Maitreyi Zaveri et al. shows that tomato extract is known to have higher inhibiting activity for tyrosinase enzyme than that of goji berry extract (Zaveri and Patel, 2012). The IC50 value of tomato is 4.08μg/ml, while goji berry has IC50 value of 5.53μg/ml. Goji berry is known for its efficacy in inhibiting tyrosinase enzyme activity and has been used as skin whitening agent in Asia. Meanwhile, ascorbic acid has also been used as a skin whitening agent with higher IC50 value of 6.4μg/ml (Zaveri and Patel, 2012).

Exposure to ultraviolet will increase proopriomelanocortin expression which is precursor for melanocyte stimulating hormone, and MSH receptor, which is TYR, Melanocortin-1 Receptor (MC1R), TYRP-1, endotelin-1 (ET-1), protein kinase C (PKC), basic fibroblast growth factor (bFGF), ACTH hormone, nerve growth hormone (NGF), steel factor, granulocyte-macrophage colony-stimulating factor (GM-CSF), hepatocyte growth factor (HGF), prostaglandin E2 and leukemia inhibitory factor (LIF). The hormones, cytokines, and growth factors

are secreted by keratinocyte cells, which then serve as paracrine signaling to stimulate melanocyte cells to produce and trigger melanin distribution (Ferreira et al., 2013). Melanin pigment synthesis process starts from hydroxylation reaction of tyrosine amino acide to 3.4 dihidroksifenilalanine (DOPA) catalyzed by tyrosinase enzyme, then DOPA will be oxidized to Dopaquinone. Vitamin C contained in tomato extract will bind copper atom located in active site of tyrosinase enzyme, thus tyrosinase enzyme becomes inactive. Dopaquinone will then bind cysteine to be cysteinyldopa which is then oxidized to pheo-melanin. In case cysteine is not bound, dopaquinone automatically forms dopachrome, and dopachrome then becomes eumelanin through decarbocylation and tautomerization processes (Hollinger et al., 2010). Polyphenol and antioxidant in tomato will be oxidized first ahead of dopa and dopaquinone, thus melanin production will be reduced. Melanin distribution will then be accelerated by PAR-2, protein activated receptor 2, as a receptor in keratinocyte cells. When PAR-2 is activated, keratinocyte cells are able to receive melanosome which contains melanin pigment synthesized by melanocyte (Baumann, 2009). Melanin may also be directly synthesized through nitric oxide (NO), which further increase cGMP content to stimulate melanin synthesis (Ferreira et al., 2013). Lycopene contained in tomato will suppress nitric oxide, thus the amount of cGMP and melanin synthesis will be reduced.

CONCLUSION

Topical tomato extract administration may reduce MDA content and melanin amount. The best result is presented by treatment group with tomato extract dose of 1.4%.

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CONFLICT OF INTEREST

There is no conflict of interest to report in this research.

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